2012 NIBIB Training Grantees Meeting

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Magnetic Resonance

Navigator-Free Self-Gated Cine MR Imaging Using 2D Cartesian Golden Step Phase Encoding

Liheng Guo, Ozan Sayin, J. Andrew Derbyshire, Daniel A. Herzka
Johns Hopkins University

PURPOSE: Cine MR imaging is important to examine certain parts of the body under motion such as the heart and the knee. Given inherent limits in MR physics, often MR imaging is not fast enough to image in real time at desired spatial resolutions. Therefore during scan, subject motion is tracked over time while imaging data is acquired continuously over multiple motion cycles. After scan, imaging data is resorted retrospectively according to the measured motion to comprise a cine at adequate spatial resolution. The central phase encode (the ky=0 PE) has been traditionally used as a projection-based navigator to measure motion in 2D Cartesian imaging, where imaging acquisition is periodically interrupted to traverse the central PE. To avoid this overhead, in this work we utilize near-center PEs for motion tracking, with added benefit of retrospectively flexible high temporal resolution of motion measurement.

METHODS: MOTION TRACKING USING NEAR-CENTER PEs: Along ky, within a limited width centered on ky=0 (the "navigation zone"), the x-inverse Fourier transform of a near-center PEs are used to track motion in the same way that the true ky=0 projection does. The temporal stream of these off-center projections ("pseudo-navigators") acquired during an ungated scan are used to extract motion for retrospective gating. GOLDEN STEP PE ORDERING: As in the original golden angle implementation, golden-step PE is used to achieve even ky and temporal coverage regardless of the width of the navigator zone. More specifically, image matrix y-size is a Fibonacci number (e.g. 233) and each PE increment by the next smaller Fibonacci number (e.g. 144). Consequently each of the 233 PE positions is covered once without repetition in 233 PEs. HUMAN SUBJECT STUDIES: 5 normal volunteers received cardiac scans (20s golden step breath-hold each) and 2 received knee scans (1-min golden step scan, during which the subject was asked to repeatedly extend and flex the knee at a comfortable pace without rhythmic cueing). Reconstruction was performed retrospectively offline.

RESULTS: Pseudo-navigator streams are clearly capable of visually depict cardiac, respiratory, and joint motion. Further, self-gated cines were successfully reconstructed at 1.2x1.2mm in-plane resolution for breath-hold non-ECG cardiac cines, and 1x1mm for free-motion knee cine. However, due to the large difference in consecutive PEs, flow and eddy-current artifacts were pronounced in cardiac acquisitions.

CONCLUSION: We have demonstrated that near-center Cartesian PEs provide adequate information for self-gating in multiple scenarios. The motion sampling rate can be freely adjusted retrospectively, permitting minimal planning before scan.

High resolution, large dynamic range measurement of MR phase maps

Joseph Dagher, Timothy Reese, Georges El Fakhri, Ali Bilgin
Massachusetts General Hospital; Harvard University

Purpose: Phase map estimation is an important step in various MR applications including fat/water separation, field mapping, chemical shift imaging, susceptibility imaging, velocity mapping, etc. In such applications, it is difficult to obtain rapid, high-resolution, and robust estimates of the underlying phase. We propose here a theory and a corresponding joint acquisition-processing solution to the problem of phase map estimation.

Methods: We formulate the problem of Minimum Variance Unbiased (MVU) estimation of phase maps as an optimization problem. The optimizer designs the acquisition such that the corresponding estimation algorithm attains an MVU phase estimate. We show that this can be achieved by acquiring the data at three optimal values of a tunable phase parameter. For example, in a field mapping application, the optimizer finds three optimal values for the echo times (TE). The optimization is run offline, once. The corresponding MR sequence acquires the images at the prescribed tuning parameters. Finally, our jointly-designed estimation algorithm combines the measurements to yield the MVU phase estimate.

Results: We first illustrate the performance of our method in field map estimation applications. We acquire Gradient Echo images at three optimal TEs, as prescribed by our optimizer. In-vivo results illustrate the advantages of our technique. For example, we were able to obtain robust, high resolution (0.8mmx0.8mmx1.5mm) field maps at 3T, acquired with a matrix size of 512x512, in a fraction of the time traditionally needed in such low SNR regime. We also show the performance of our method in acquiring high resolution chemical shift maps at 7T. The resulting maps exhibit large dynamic range without phase wrapping artifacts. Wrapping artifacts are a particularly hindering challenge in many phase mapping applications. Our method overcomes these artifacts elegantly. Finally, we validate our method quantitatively using a water/oil phantom.

Conclusion: We have presented here a novel phase map estimation method which consists of a practical, intuitive and easy to implement acquisition-processing procedure. The main advantage of our method is its ability to rapidly and robustly estimate phase maps over a large dynamic range without requiring the use of phase unwrapping procedures. This is a key property which allows us to overcome both noise and hardware limitations.

Magnetization Spoiling in Radial FLASH Contrast-Enhanced MR Digital Subtraction Angiography

Parmede Vakil, Sameer A. Ansari, Michael C Hurley, Himanshu Bhat, Timothy J. Carroll
Northwestern University, Dept. of Biomedical Engineering

Introduction: In CE MRA, for a given FOV, there is a tradeoff between SNR, spatial resolution, and temporal resolution. A high image update rate (> 1 frame/second) is necessary for visualizing complex flow patterns in brain AVMs. At the same time, high SNR and spatial resolution are required for small vessel discrimination (< 1 mm). Currently, many 3D protocols have been developed using various under-sampling schemes in Fourier space which serve to increase temporal resolution at the expense of SNR. On the other hand, there are only few reports on 2D acquisitions in CE MRA. When used in conjunction with a static, high resolution MRA, however, 2D acquisitions provide a comprehensive exam. In this study we present a novel 2D MRI pulse sequence that produces high frame rate (6 frames/sec) CE MR Angiograms and sufficient SNR for small vessel discrimination that can be used in the setting of intracranial vascular malformations.

Purpose: To increase the in-plane spatial resolution and image update rates of 2D magnetic resonance (MR) digital subtraction angiography (DSA) pulse sequences to 0.57 x 0.57 mm and 6 frames/sec, respectively, for intracranial vascular disease applications by developing a radial FLASH protocol and to characterize a new artifact, not previously described in the literature, which arises in the presence of such pulse sequences.

Materials and Methods: The pulse sequence was optimized and artifacts were characterized using simulation and phantom studies. With Institutional Review Board (IRB) approval, the pulse sequence was used to acquire time-resolved images from healthy human volunteers and patients with x-ray DSA-confirmed intracranial vascular disease.

Results: Artifacts were shown to derive from inhomogeneous spoiling due to the nature of radial waveforms. Gradient spoiling strategies were proposed to eliminate the observed artifact by balancing gradient moments across TR intervals. The resulting radial 2D MR DSA sequence (2.6 sec temporal footprint, 6 frames/sec with sliding window factor 16, 0.57 x 0.57 mm in-plane) demonstrated small vessel detail and corroborated x-ray DSA findings in intracranial vascular imaging studies.

Conclusion: Appropriate gradient spoiling in radial 2D MR DSA pulse sequences improves intracranial vascular depiction by eliminating circular banding artifacts. The proposed pulse sequence may provide a useful addition to clinically applied 2D MR DSA scans.

Use of Real Time MRI for Measurement of Post-Infarct Cardiac Remodeling via Load-Independent Indices

Francisco Contijoch, Walter RT Witschey, Jeremy R McGarvey, Melissa M Levack, Gerald A Zsido, Manabu Takebe, Norhiro Kondo, Christen Dillard, Joseph H Gorman III, James J Pilla, Robert C Gorman

University of Pennsylvania

Purpose: Conventional clinical trial endpoints of heart disease, left ventricular volumes (VLV) and ejection fraction (EF), are viewed through the prism imposed by cardiac inotropic state. There is a need for reproducible, regional and simple cardiac endpoints that are independent of or can measure the relative inotropic state. The emergence of real time magnetic resonance imaging (MRI) approaches may soon allow noninvasive measure of regional end-systolic and end-diastolic pressure volume relations

Methods: The MR imaging protocol consisted of short-axis, cine-bSSFP and golden angle radial bSSFP MRI performed on a clinical 3 T MRI scanner. A custom bSSFP, golden angle radial acquisition was coordinated with an inflow occlusion to capture baseline and occluded heart beats. LV pressure was obtained from the catheter placed in the LV cavity. Slice volumes were segmented from cine-SSFP and real time short axis images in ITK-Snap. Real time short axis images were segmented in ITK-SNAP to identify endocardial and epicardial boundaries. For 3 beats during the experiment, 16 points along the endocarial and epicardial surfaces were identified. Non-rigid registration of the end-diastolic image to subsequent images in the heartbeat was used to calculate the cardiac motion.

Results: The inflow occlusion was performed within a 90 second MRI acquisition. The real time pressure-volume loops are very similar in shape and calculated ejection fraction to the traditional CINE method. Furthermore, the occlusion allows for estimation of ESPVR and EDPVR and calculation of Vo. Results indicate that a nonlinear fit of ESPVR results in improved estimates of Vo. Transient preload reduction resulted in significant alterations in regional myocardial wall strain. Strain was reduced 47% (anterior wall) and 36% (septal and lateral) in the first 3 sec of inflow occlusion. The values were reduced by 62% (anterior), 61% (septal), 57 % (lateral) 30 s later in the occlusion. Notably, changes in regional wall motion were observed in otherwise normal myocardial tissue, basal to infarcted myocardial segments.

Conclusions: Load independent indices of contractility such as ESPVR and V0 can be measured using real-time MRI during an inflow occlusion. Furthermore, the method is sensitive to the nonlinearity of the ESPVR and allows for an appropriate estimate of V0 which is difficult with conductance catheters. Regional variations in local myocardial strain were observed during transient preload reduction using a real time magnetic resonance imaging method. These findings provide insight about load-dependent changes in contractile function in post-infarction LV remodeling.

Molecular Correlates to in vivo Hyperpolarized [1-13C] Dehydroascorbate Reduction

Victor Sai, Kayvan Keshari, Romelyn Delos Santos, John Kurhanewicz, David M. Wilson
University of California, San Francisco

Purpose: To correlate the high hyperpolarized (HP) 13C-Vitamin C signals observed by MR spectroscopic imaging (MRSI) in the tumors of TRAMP (transgenic model of prostate cancer) mice with transporter expression, intracellular glutathione concentration, and enzymes involved in regulating redox.

Methods: In vivo MRSI studies using HP [1-13C] DHA were performed as previously described (Keshari et al. 2011). DHA and Vitamin C resonance heights were used to calculate average metabolite ratios (VitC/ [VitC + DHA]) for voxels corresponding to both tumor and surrounding benign tissues in TRAMP mice (n=4). Intracellular glutathione and thioredoxin reductase concentrations were measured on homogenized prostate tissue lysates via a DNTB based assay. Real-time PCR was performed on total RNA isolated from frozen tissue extracts of TRAMP mice and normal prostate controls. Mercury orange staining and fluorescent microscopy were performed on mouse prostate tissue sections. Immunohistochemistry with GLUT1 and thioredoxin reductase antibodies was performed per standard protocol.

Results: TRAMP tumors demonstrated elevated ratios of HP vitamin C to DHA (VitC/[VitC + DHA] = 0.31± 0.05) compared to surrounding normal tissues and benign prostate (VitC/[VitC + DHA] = 0.15 ± 0.03) (n=4). The level of non-protein thiols observed on mercury orange staining was elevated both qualitatively and quantitatively; mean fluorescent intensity averaged over three ROIs was 1108 ± 67 in TRAMP and 776 ± 56. Intracellular glutathione levels were significantly elevated in TRAMP prostate (5.8 mM ± 1.2) compared to normal prostate (1.1 mM ± 0.2). Thioredoxin reductase levels were also significantly elevated in TRAMP prostate (0.64 µmol/min/mL ± 0.26) vs. normal prostate (0.14 µmol/min/mL ± 0.12). The relative expression of GLUT1 in TRAMP was comparable to normal prostate (4.8 relative % expression to M. Cyclophilin compared to 5.2, respectively), but GLUT3 and thioredoxin reductase expression levels were increased (GLUT3: 1.2 relative % expression compared to 0.1; thioredoxin reductase: 7.0 relative % expression compared to 1.0). Immunohistochemistry demonstrated increased staining of TRAMP tumors with thioredoxin reductase compared to normal prostate (63% vs. 9%), but GLUT1 staining was not significantly different.

Conclusion: Marked reduction of [1-13C] DHA to [1-13C] vitamin C in vivo in TRAMP is likely dependent on elevated levels of intracellular glutathione and thioredoxin reductase as well as extracellular thiols. This suggests that HP [1-13C] DHA MRSI may be a noninvasive method to assess the antioxidant phenotype of tumors, which has been shown to correlate with increased aggressiveness and resistance to therapy.

In Vivo Hyperpolarized Spectroscopy of Hypoxia Inducible Factor-1 Activity in Murine Sarcoma

Ryan M Davis, Ruby Qiu, Minsi Zhang, David Kirsch, Warren S Warren
Duke University

Background: For almost a century, scientists have noticed that cancer cells tend to produce more lactic acid (LA) than normal cells, a tendency which has been termed the "Warburg effect". Now, it is well known that LA levels are determined not only by mass action relations between glucose, oxygen, ATP, and LA, but also by a concerted effort of dozens of metabolic enzymes that are actively regulated by genes and their corresponding proteins. One gene/protein pair that regulates metabolic enzymes and has received much attention is hypoxia inducible factor-1 (HIF1). HIF1 has been shown in in vitro (cell culture) experiments to regulate enzymes involved in LA production such as glucose transporters and lactate dehydrogenase (LDH). The observation that HIF1 regulates cancer cell metabolism and lactate production in vitro raises two important questions. First, to what extent does HIF1 regulate LA production in vivo? Second, is it possible to measure LA production in humans as a biomarker of tumor stage or progression? Fortunately, nuclear magnetic resonance offers a set of tools extremely well suited to answer both of these questions.

Methods: A murine sarcoma model was imaged with a 300MHz Bruker Biospec magnetic resonance imaging system. Wild-type and HIF1 knockout mice were imaged. Lactate spectral editing and hyperpolarized pyruvate 13C imaging are used to measure lactate levels and lactate dehydrogenase activity respectively for wild type and knockout mice.

Initial Results: The integral of lactate edited signal was linearly proportional to actual lactate concentration (4mM – 10mM) in phantoms ($R2 = 0.9971$). The lactate editing pulse sequence suppressed the lipid signal to < 3% which is sufficient. The integral of the lactate peak in the in vivo lactate edited spectra divided by the standard deviation of the noise level (i.e. the SNR) ranged from 37-75, depending on the mouse. In a separate set of experiments, hyperpolarized 13C-pyruvic acid was converted to LA within seconds after pyruvic acid injection.

Conclusion: We have implemented imaging and spectroscopy experiments enabling measurement of LA and LDH activity in vivo. We are currently using the platform to test two hypotheses. First, steady state lactate levels (measured with lactate spectral editing) are lower in HIF1 knockout mice than in wild-type mice. Second, the real-time conversion of pyruvate to lactate (measured with hyperpolarized 13C-pyruvate imaging) is slower in HIF1 knockout mice than in the wild-type mice.

Chemical Exchange Saturation Transfer (CEST) Imaging of the Spinal Cord at 7T

Feliks Kogan, Anup Singh, Mohammad Haris, Kejia Cai, Hari Hariharan, Ravinder Reddy
University Of Pennsylvania

Introduction: Glutamate (Glu) is the primary neurotransmitter responsible for excitatory synaptic transmission in the brain stem and spinal cord. In this study, we describe a novel, noninvasive approach for imaging glutamate in the spinal cord by exploiting the chemical exchange of protons between the amino group (-NH2) of Glu and water using a technique known as chemical exchange saturation transfer (CEST).

Methods: A single voxel proton MR spectrum (SVS) from a bovine cervical spine specimen was acquired using a STEAM sequence and 100 signal averages. The CEST effect from all major metabolites present in the spinal cord as observed with MRS were imaged under physiological conditions in a phantom. Finally, CEST imaging was performed on a healthy 26 year old human cervical spinal cord. Two signal-averaged acquisitions were performed for calculating CEST maps. All CEST images and glutamate CEST contrast maps were corrected for B0 and B1 inhomogeneities.

Results and Discussion: Using the experimental parameters to optimize the Glu CEST contrast, glutamate is responsible for most of the CEST effect. A 6% CEST effect was observed from 10 mM glutamate with small contributions from glycine (~0.4 %) and Cr (~0.8%), and negligible contributions from all other metabolites visible with MRS. These results demonstrate that with these particular parameters, the majority of the CEST contrast is due to glutamate. The glutamate CEST map obtained from a healthy human cervical spinal cord demonstrates a distinct gray (GM) and white (WM) matter distribution pattern (figure 3). The average CESTasym in the GM was 6.0% compared to 4.1 % in the WM. A minimal CEST signal (0.8±0.7%) was observed in the cerebrospinal fluid (CSF) indicating that B0 inhomogeneities, which are a major concern for CEST imaging at 7T, were adequately corrected for. The findings of this preliminary study suggests that the in vivo high resolution mapping of Glu is feasible using the CEST technique, which provides a new method to detect changes in Glu concentration in spinal cord disorders. Conclusion: In this work, we demonstrated that it is feasible to detect the CEST effect from glutamate at 7T with high spatial resolution. We showed that with the optimal parameters for Glu CEST contrast, the majority of the CEST effect is due to Glu with minor contributions from Cr and Gly. CEST contrast from glutamate can be used to study relative distribution and any changes in Glu in the spinal cord in vivo.

A Novel Approach for Global Noise Reduction in Resting-State fMRI: APPLECOR

Michael Marx, Kim Butts Pauly, Catie Chang
Stanford University

Purpose: Noise in fMRI recordings creates uncertainty when mapping intrinsic connectivity networks in the brain. Correlated physiological noise can altering the apparent strength or extent of intrinsic networks. The purpose of this work was to develop a data-driven noise correction that could achieve superior noise removal for resting-state fcMRI.

Methods: The new correction is termed APPLECOR, for Affine Parameterization of Physiological Large-scale Error CORrection. APPLECOR models common physiological noise as the linear combination of an additive term and a mean-dependent multiplicative term, and then estimates and removes these terms. APPLECOR was also combined with RVHRCOR to create PEARCOR. One metric used to validate the new correction was consistency of the default mode network (DMN) after correction. Four corrections were applied to 15 resting-state fMRI recordings. Correlation between voxels in the brain and voxels in the posterior cingulate cortex (PCC) were measured before and after each of the corrections. Temporal consistency was measured by dividing each 16-min data set into smaller (2-min) time windows and analyzing the variation of the DMN across these windows. Inter-subject consistency was measured as variation in a normalized space.

Results: Averaged across all gray and white matter voxels across all subjects, APPLECOR and PEARCOR improved average temporal consistency by 9% and 11% over raw results, as compared to the other corrections that gave a 2% and 5% improvement. Spatial consistency of the temporal variation was improved by 11% and 19% with the new corrections, as compared to 5% and 7% with the alternative corrections. APPLECOR and PEARCOR improved inter-subject consistency by 16% and 18% over raw results, as compared to 6% and 15% for the alternative corrections. Spatial consistency of the inter-subject variability was improved by 2.4% and 4.1% with the new corrections versus 1.8% and 3.3% for the alternative corrections. The new corrections greatly reduced the appearance of “false positives” in apparent DMN connectivity within the smaller time windows.

Conclusions: Two new corrections, APPLECOR and PEARCOR, have been developed, and their properties have been analyzed using functional connectivity analysis of the default mode network. The two corrections achieved greater consistency of the default mode network across time and across subjects than the alternate corrections compared here. Without knowing the ‘true’ functional connectivity of the brain, validation of any data-driven noise reduction procedure is challenging; yet, the increased consistency of the DMN topology supports the efficacy of the proposed model.

Single Cardiac Cycle Multipoint T1 Mapping with Radial Acquisition

David Chen, Behzad Sharif, Rohan Dharmakumar, Debiao Li
Northwestern University

Background: Measurement of myocardial blood flow by MRI is complicated by nonlinearity of signal intensity to contrast agent concentration. This will cause underestimation of myocardial blood flows.

Purpose: To use a radially acquired MR technique to directly measure contrast agent concentration.
Methods: Healthy volunteers underwent 3 first pass rest perfusion scans using a radially acquired MR scan, a normal TI Cartesian scan, and a short TI Cartesian scan. The AIF was found using an ROI placed in the LVBP. Signal saturation was corrected by finding T1 maps each frame using radially acquired data. Corrected AIF was compared with the short TI Cartesian scan, which is insensitive to signal saturation.

Results: The normal TI Cartesian scan shows significant truncation due to T1 recovery signal saturation. The short TI Cartesian scan yields a higher peak amplitude because image acquisition starts before magnetization recovers significantly. Similar to the long TD Cartesian scan, the uncorrected radial AIF shows truncation due to signal saturation. The proposed T1 mapping technique allows for correction of the radial AIF.

Conclusion: Contrast concentrations were found by measuring T1 on a frame by frame basis. The described method eliminates the need to perform two scans or acquire two datasets to produce an unsaturated AIF, increasing feasibility for clinical use.

Magnetic Resonance Thermometry at 7T for Real-Time Control of Ultrasound Induced Mild Hyperthermia

Brett Z. Fite, Yu Liu, Dustin E. Kruse, Charles F. Caskey, Jeffrey H. Walton, Chun-Yen Lai, Lisa M. Mahakian, Benoit Larrat, Erik Dumont, Katherine W. Ferrara
University of California, Davis

Purpose: To evaluate the feasibility of magnetic resonance thermometry (MRT) at 7T for real-time control of ultrasound-induced hyperthermia in small animal models.

Methods: Five MR temperature measurement sequences (GRE with and without GRAPPA, segmented EPI with and without GRAPPA) were validated against a fluoroptic temperature probe in a tofu phantom using the Bruker 70/30 Biospec 7T MR imaging system, Image Guided Therapy ultrasound insert and custom image reconstruction and temperature estimation software. The signal-to-noise ratio and the spatial, temporal, and thermal resolutions of the sequences were compared in vitro. Real time control of temperature was achieved via a proportional, integral, derivative (PID) controller, which used MR-derived temperature maps as its input and from which the output dictated the acoustic power delivered during a single shot. The stability of the controller was examined in a tofu phantom for a temperature elevation and time course consistent with mild hyperthermia protocols. Four Met-1 tumor bearing female FVB mice were insonated under real time temperature control to evaluate the performance of the MRgFUS system in vivo. All animal studies were approved by the UC Davis Institutional Animal Care and Use Committee.

Results: All sequences examined yielded a temperature precision of less than 1 °C even with a receive volume coil and with temporal resolution as low as 100 ms and spatial resolution of 1 mm3. The utilization of a phased array receive coil (4-channel rat brain phased array, Bruker Biospin, Ettlingen, Germany) in conjunction with the GRAPPA algorithm improved image contrast and temporal resolution without significantly decreasing thermal precision. In a tofu phantom under real time feedback control, the desired temperature was maintained within 0.5 °C of the desired temperature for 15 minutes. In vivo, a requested local temperature increase at the tumor agreed within 0.27 ± 0.15 °C when compared to the animals' starting core body temperature. However, due to global heating from heat diffusion, the requested 4 °C local increase yielded a measured increase of 3.1 ± 0.4 °C at the tumor when compared to the animals' core body temperature in real time.

Conclusions: The developed MRgFUS system has demonstrated the ability to deliver a controlled thermal dose under real time PID control both in a tofu phantom and in vivo in a Met-1 mouse tumor. PPI acquisition with GRAPPA based reconstruction demonstrated adequate thermal accuracy, high temporal resolution, and was successfully implemented within the experimental MRgFUS system.

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Quantifying Myocardial Fibrosis in Hypertensive Left Ventricular Hypertrophy using T1 Mapping

Rajesh Janardhanan, Nebiyu Adenaw, Ronny S. Jiji, Jeremy Brooks, Frederick H. Epstein, Christopher M. Kramer, Michael Salerno
University of Virginia

BACKGROUND: Diffuse myocardial fibrosis can occur in hypertensive left ventricular hypertrophy (LVH) and is not readily detected by conventional late gadolinium enhanced CMR. We previously described a shortened Modified Look-Locker Inversion Recovery (3-5 MOLLI) T1-mapping technique which can quantify fibrosis by calculating the partition coefficient (?) and volume of distribution (Vd) of gadolinium (Gd) following a bolus injection of Gd.(1) We hypothesized that this technique could detect fibrosis in subjects with hypertensive LVH and normal ejection fraction. This could have important implications for assessment of diastolic heart failure and to monitor benefits of anti-fibrotic, anti-hypertensive therapy. We aimed to detect diffuse myocardial fibrosis in hypertensive patients with LVH as compared to age matched normal controls.

METHODS: T1 mapping was performed in 11 subjects with hypertensive LVH (53±16 years) and normal ejection fraction, and 7 age-matched healthy volunteers (50±10 years) on a Siemens 1.5T Avanto using 3-5 MOLLI (11 heart beats, 2 inversions, 3 recovery beats, 8 images). Patients with known coronary disease, significant valvular disease, and other causes of LVH were excluded. LV mass and function was assessed by SSFP cine imaging. MOLLI sequence parameters included: TE/TR/FA 1.1 ms/2.5ms/35°, FOV= 340 x 260, resolution 1.8mm x 1.8mm, thickness 8mm. T1 was determined pre-contrast and 10,15 and 20 minutes following injection of 0.15 mmol/kg Gd-DTPA. Hematocrit (Hct) was measured in all subjects. T1 maps were calculated and manually segmented using an in-house MATLAB program. ? was determined from the slope of a plot of 1/T1 of the myocardium versus 1/T1 of the blood. Vd was calculated as (1-Hct)*?. Values were compared between groups using 2-tailed unpaired t-tests.

RESULTS: The LVH group had significantly higher blood pressure and LV mass than age-matched controls. Heart rate, Hct and creatinine were similar between groups. Values for ? and Vd were higher in LVH (0.49±0.04 and 0.31±0.02) than controls (0.44±0.01 and 0.27±0.01) (p=0.003 and 0.004) respectively. There was a positive association between LV mass and ? (Spearman rho=0.66; p=0.01).

CONCLUSIONS: Determination of ? and Vd by T1 mapping after Gd bolus with a reduced breath-hold 3-5 MOLLI pulse sequence is a robust method capable of quantifying diffuse myocardial fibrosis in hypertensive patients with LVH and normal ejection fraction. ? correlates with LV mass. These findings support the application of T1 mapping to monitor therapies that regress hypertrophy and reduce fibrosis in hypertensive heart disease.

(1) Janardhanan et al. JCMR 2011,13:O81

Manganese--Enhanced MRI in the Evaluation of Cell--Based Therapy

Paul Kim, Ildiko Toma, Yongquan Gong, Phillip Harnish, Phillip Yang
Stanford University

Background : To date, the underlying mechanism responsible for the restoration of the injured myocardium following transplantation of stem cells has not been clearly identified. Three major hypotheses have been previously proposed: cardiac differentiation of transplanted cells (de novo myocardial regeneration), paracrine effect on existing myocardium (myocardial salvage) or recruitment of cardiac progenitor cells (resident stem cells). Manganese-enhanced MRI (MEMRI) allows a reliable method of imaging viable myocardium. Utilizing MEMRI, we evaluated the changes in the viability of the injured myocardium to further investigate the underlying mechanism of functional restoration using stem cell therapy.

Methods: Thirteen Fox Chase SCID Beige mice were subjected to permanent left anterior descending (LAD) ligation to create a mouse myocardial injury model. 2.5 x 105 reporter-gene transduced mouse embryonic stem cells (ESC-RGs) containing firefly luciferase (fluc) were transplanted into the intra-infarct region in 11 mice. Two mice were injected with normal saline into the intra-infarct region to serve as controls. 3T cardiac MRI was performed weekly for 4 weeks following LAD ligation and ESC-RGs transplantation to obtain LVEF measurements and MEMRI images. Additionally, bioluminescence images (BLI) were obtained weekly utilizing the transduced fluc gene to demonstrate persistent viability of the ESC-RGs. At weeks 2, 3 and 4, the hearts were explanted, sectioned along the short axis plane and processed for H&E staining. The H&E stained slides provided histological correlation of MEMRI and BLI.

Results: We demonstrate a trend towards improved LVEF with ESC-RGs transplanted hearts, consistent with the results of our group's previously published data. The control group, in contrast, demonstrates no functional improvement with a persistently depressed LVEF. A more sensitive measurement of myocardial restoration is significantly increased MEMRI signal observed in the ESC-RGs vs. control mice (.119+.005 cm3 vs .0736+.001 cm3 respectively, p=0.034), indicating improved myocardial viability (Figure 1). BLI confirmed the presence as well as engraftment of the transplanted ESC-RGs, which were confirmed histologically (Figure 2).

Conclusions: This study demonstrates the functional improvement in ESC-RG transplanted mice. In addition, MEMRI shows a significant increase in viable myocardium in ESC-RG transplanted hearts. This finding may support the hypothesis that functional restoration with stem cell therapy may be due to increased viability of the myocardium.

Feasibility of Quantitative 31P NMR Imaging of Cortical Bone

Alan C. Seifert, Alexander C. Wright, Cheng Li, Suzanne L. Wehrli, Felix W. Wehrli
University of Pennsylvania

Introduction and Purpose: Current diagnostic bone density exams are x-ray based, and can only measure bone mineral. MRI has the potential to image and quantify both mineral and matrix densities; this combination has the potential to give more information on bone health. With ultra-short echo time imaging methods currently used by our group and others, we hypothesize that phosphorus in cortical bone mineral can be imaged and quantified using MRI.

Methods: Two MRI pulse sequences which are suitable for imaging short-T2* solid-state phosphorus are ultra-short echo-time (UTE) and zero echo time (ZTE). In UTE, a short, non-selective RF pulse is applied, and then after the receiver dead-time, imaging gradients are turned on and data acquisition is started. In ZTE, the imaging gradients are turned on and allowed to stabilize, then a short RF pulse is applied. After the receiver dead-time, acquisition is started. ZTE captures each k-space point sooner after excitation, but due to receiver dead time, some points in the center of k-space are lost, and this central region must be refilled by some other method, thus complicating reconstruction. Signal-to-noise ratio usually increases with field strength in MRI, but because the relaxation properties of solid-state phosphorus become dramatically less favorable at high field, it was necessary to measure the T2* and T1 relaxation times at multiple field strengths by line-width and saturation-recovery measurements, respectively. These times were incorporated into the gradient-echo signal equation, which was used to estimate the achievable SNR at each field. By imaging bone with a reference sample of known phosphorus density and correcting for relaxation and RF coil inhomogeneity, it is possible to measure bone mineral density by comparing the bone signal intensity to that of the reference.

Results: ZTE images have higher SNR than UTE images. T2* decreases (220μs at 1.5T to 98μs at 11.7T) and T1 increases dramatically (12s at 1.5T to 97s at 11.7T) as field strength increases, but predicted SNR does increase at higher field strength.

Conclusion: Despite more complicated reconstruction, ZTE's higher intrinsic SNR and lack of FOV restriction to imaging gradient isocenter will likely render it superior to UTE. Because phosphorus only exists in high concentration in bone, image blurring due to broad line width can be managed by attributing phosphorus signal outside the bone to the bone itself, rather than surrounding soft tissue. These results suggest that future in vivo quantification of bone mineral density should be feasible.

Advanced MR Molecular Imaging through Contrast Modulation of Contrast Agents

Bo Zhu, Thomas Witzel, Bruce Rosen, Lawrence Wald
Massachusetts Institute of Technology

Purpose: Typical magnetic resonance contrast agent imaging methods (T1, T2/T2*) have difficulty separating the image signal due to injected contrast agents from the signal due to background tissue. The predominant strategy for locating these agents involves a manual comparison of pre- and post- injection images. However, biological uptake of targeted contrast agents often occurs in tens of minutes or hours, during which confounds such as subject movement and extraneous biological 1/f noise are introduced into the pre/post image difference, often obfuscating the signal change due to the agent. We propose Acoustically Induced Rotary Saturation (AIRS), an entirely post-injection method for selectively detecting contrast agents based on narrowband detection of oscillating magnetic fields generated by vibrating contrast agent nanoparticles.

Methods: A rotating frame resonance condition (i.e. the Rotary Saturation effect) is established between the spin-locked water magnetization and the oscillating magnetic fields generated by mechanically or acoustically vibrated contrast agents, whereby the image signal is saturated only when the agents' vibration frequency is tuned to the spin-lock resonant frequency. We validate the ability to "activate" or "deactivate" the contrast agent effect with a block-design imaging experiment on two phantom setups, switching the contrast agent vibration frequency on- and off-resonance with the spin-lock condition. The first setup used a Gd-doped liquid water phantom, within which a glass capillary tube containing an aqueous sample of Fe2O3 nanoparticles was vibrated by a piezoelectric bending actuator. The second phantom setup was a tissue-mimicking agar-gelatin phantom with Fe2O3 agar-gelatin phantom inserts; the entire gel was vibrated from its top surface with a piezoelectric bending actuator. We characterize the AIRS effect (relative signal change ?S/S) across vibrational displacement (10 to 500 μm), resonant frequency (50 to 200 Hz), and Fe concentration (50 to 200 μg/ml).

Results: Statistical analysis of images from the block-design experiment using standard fMRI techniques (FEAT/FSL) reveals statistically significant signal changes appear only in the vicinity of the Fe2O3 nanoparticle sample, effectively creating an "activation map" of the contrast agent. Characterization experiments demonstrate a nearly linear relationship between ?S/S and vibrational displacements as well as contrast agent concentration, but no change across resonant frequency.

Conclusions: AIRS is capable of selectively imaging iron oxide contrast agents by modulating their contrast through a resonant spin-lock mechanism, validated in both liquid and gel phantoms. Our characterization experiments show the AIRS effect to be generally well-behaved and robust across contrast agent concentration, displacement, and operating vibration frequency.

Exercise/rest calf muscle perfusion and perfusion reserve using contrast-enhanced MRI in PAD

Ronny S. Jiji, Amy W. Pollak, Frederick H. Epstein, Patrick F. Antkowiak, Craig H. Meyer, Arthur Weltman, Joseph M. DiMaria, Jennifer R. Hunter, John M. Christopher, Christopher M. Kramer
University of Virginia

Purpose: To determine whether perfusion or perfusion reserve with first-pass contrast enhanced MRI is the most reproducible measure of exercise calf blood flow.

Methods: Sixteen healthy subjects and 3 symptomatic PAD patients (ABI 0.4-0.9) underwent contrast-enhanced perfusion MRI of the calf before and after plantar-flexion exercise using an MR-compatible pedal at 50 rpm for up to 20 minutes or until limiting symptoms. Contrast-enhanced first-pass images were obtained on a 3T Siemens Trio scanner by infusion of 0.1mM/kg of gadolinium-DTPA followed by a 20 mL saline flush at 4 mL/second. A spoiled gradient echo dual contrast sequence with slices positioned 32 mm apart allowed for simultaneous acquisition of arterial input and muscle perfusion images. Time-intensity curves (TIC) were generated using ARGUS software (Siemens) for arterial input and the muscle group with the highest signal intensity (tissue function, TF). PI was measured as ratio of the slopes of the TF TIC/arterial input TIC. PR was defined as exercise TF/resting TF. PIR was calculated as exercise PI/rest PI. Ten of the controls and all 3 patients underwent repeat studies on a separate day. For 7 controls and all patients, measurements were normalized to proton density.

Results: Participant mean age was 60±8. ABI in PAD was 0.70±0.11. Exercise time was 816±453 seconds for normals and 567±516 seconds for PAD. The anterior tibialis commonly demonstrated the highest signal intensity. Median exercise PI and rest PI were 0.29 (25th, 75th percentiles = 0.22, 0.52) and 0.018 (0.009, 0.052) in 16 healthy subjects. Mean PR and PIR for normal subjects were 30±20 and 21±17, respectively. For normal and PAD patients with repeat studies, intraclass correlation (ICC) for exercise PI was the same for both normalized and non-normalized measurements (0.73). The ICC for rest PI improved with normalization (0.64 vs 0.77). The ICC for PR and PIR were weaker than for PI.

Conclusions: Exercise PI remains the most reproducible measurement for quantification of exercise calf perfusion and does not require signal normalization under these experimental conditions. Perfusion reserve measurements are less reliable, likely due to the inherently lower values for arterial input and tissue function at rest and resultant variability.

Abstract Summary Statement: Of the parameters available to quantify exercise calf perfusion by first-pass contrast- enhanced MRI, perfusion index is the most reproducible method and does not require normalization to proton density

High temporal resolution quantification of global CMRO2 during apneic challenge

Zach Rodgers, Varsha Jain, Michael Langham, Jeremy Magland, Felix Wehrli
University of Pennsylvania

PURPOSE: CMRO2 (cerebral metabolic rate of oxygen consumption) is an important index of cerebral metabolism altered in many neurological diseases. Faster CMRO2 quantification is needed to better understand the temporal dynamics of neurovascular coupling in health and disease. In this study, a CMRO2 quantification method with 2.5-second temporal resolution is developed and validated during a breath hold paradigm.

METHODS: A multi-echo GRE pulse sequence was developed to simultaneously measure total cerebral blood flow (tCBF) and venous oxygen saturation (SvO2) in cerebral blood vessels. By selecting an oblique axial slice rotated 15 degrees in the coronal plane, the internal carotid arteries, basilar artery, and superior sagittal sinus (SSS) can be captured in one slice. Flow velocity is quantified from the phase difference between images acquired with positive and negative first gradient moment at the first of three echos. SvO2 is quantified from the phase accumulation in the SSS between the first and third echo, which scales with SvO2 due to the paramagnetic properties of deoxyhemoglobin. Acquisition time is reduced by acquiring only the middle fourth of k-space and using keyhole reconstruction to fill outer k-space from a fully-sampled reference image acquired before the main sequence. CMRO2 is quantified by Fick's principle as the product of tCBF and the arterio-venous difference, normalized to brain volume and hematocrit: $CMRO2=Ca \bullet tCBF \bullet (SaO2-SvO2)$. SaO2 is measured with pulse oximetry, brain volume measured with a 3D MPRAGE image set, and hematocrit assumed as 0.4. A healthy 26 y.o. subject was scanned on a 3T Siemens Trio scanner using a 12-channel head and 2-channel neck coil during a paradigm involving two 30s breath holds with 90s recovery.

RESUTLS: CMRO2 averaged over the initial 90 s baseline was 118±6 μmolO2/100g/min, in agreement with literature. Flow and SvO2 increased during breath hold and dropped during recovery, undershooting before returning to baseline. CMRO2 remained relatively constant during breath-hold, dropping during recovery before returning to baseline. Interestingly, SSSBF increases about twice as much relative to baseline than tCBF (105% vs. 48%). Therefore, calculating CMRO2 with SSSBF substituted for tCBF changes the observed CMRO2 response significantly, causing a marked increase during breath-hold with return to baseline during recovery.

CONCLUSIONS: This study indicates the feasibility of using keyhole susceptometry and velocitometry to increase the temporal resolution of CMRO2 quantification to 2.5s, enabling observation of the temporal dynamics of cerebrovascular coupling. The method is currently being further validated in a cohort of 10 healthy subjects.

Optical Imaging

Custom multiphoton microscope for long-term imaging of neurons under chronic closed-loop stimulation

W. Alexander Calhoun, Ushnik Ghosh, Eric Eisner, Sam Rakowski, Steve M. Potter
Georgia Institute of Technology, Dept. of Biomedical Engineerng

Neurons in vitro exhibit characteristic bursting behavior, resembling epileptic seizures, hypothesized to be the result of homeostatic response to loss of synaptic inputs. Closed-loop electrical stimulation has been shown to reduce or eliminate these bursts over several days. However, little is known about the extent to which this long-term stimulation can induce morphological or synaptic changes in the network, or the time course over which any such changes occur. We will use multiphoton microscopy to study the dynamics of neurons under chronic stimulation in detail. We have developed a custom multiphoton system capable of imaging living neurons continuously for days with concurrent electrical recording and stimulation through a microelectrode array (MEA).

Long-term microscopy of living cells poses several challenges. First, the cells must be kept alive and healthy for the duration of the experiment through careful maintenance of temperature, pH, and osmolality. Second, care must be taken to minimize unwanted light exposure and fluorescence excitation, which are detrimental to cell viability over long periods of time. Finally, the imaging system must autonomously maintain multiple independent subsystems that must be working properly to ensure constant image quality.

We have developed a flexible, inexpensive microscope incubation system which can maintain an optimum environment for living cells in vitro. Cultures are grown on MEAs sealed with semi-permeable Teflon lids, which maintain sterility and osmolality. This allows us to image cultures continuously for days. While the selective excitation inherent to multiphoton microscopy reduces phototoxicity, exposure to intense infrared excitation light can cause local heating and non-linear photodamage. We use multiple collection paths to capture multichannel fluorescence and brightfield images in a single pass, then use analog integration to efficiently detect emitted light, reducing the excitation required. We are also working on closed-loop control of excitation power to improve image stability for very long experiments.

We have developed an autonomous monitoring system for discrete microscope components using an Arduino microcontroller, which tracks environmental conditions, laser output, and other critical system parameters. It can provide status reports to acquisition software or shut down the system in case of an emergency, such as loss of laser cooling.

Our system is capable of imaging live cells for several days without interruption. We have begun using it to study morphological and functional plasticity in living neurons using genetically encoded fluorescent proteins. Further work is needed to extend to weeks of uninterrupted imaging, such as perfusion systems for media changes.

Imaging the Initiation of Inflammation in the Brain with Intravital Two-photon Microscopy

Deborah Sim Barkauskas, Jay Myers, Alex Huang
Case Western Reserve University

Purpose: Experimental autoimmune encephalitis (EAE) is a mouse model of multiple sclerosis. Limitations in current techniques to capture rare immune cell events in space and time have hindered the characterization of the initiation and progression in EAE. Advances have been made in our ability to visualize these processes on the cellular level with the advent of two-photon microscopy and the cranial window technique we are able to serially image the brain over time with minimal disruption to the tissue environment. We show that brain resident immune cells, microglia, and infiltrating antigen presenting cells (APCs) are responsible for the infiltration of myelin specific T cells into the brain parenchyma across the blood brain barrier.

Methods: We use CD11c-GFP or CX3CR1-GFP and ubiquitin-CFP myelin oligodendrocyte glycoprotein (MOG) specific transgenic mice. A craniotomy was sealed with a #1 cover glass bonded to the skull with vetbond. Dental acrylic was used to create a permanent well to maintain the fluid needed with an immersion objective. 4 days after the surgery, 3x106 myelin specific T cells were injected intravenously into the mouse. 24 hours later, EAE was induced via peripheral immunization with MOG-peptide admixed in CFA, and the progression of disease was imaged for 1 hour each day over the first 12 days of disease. Volumes of 775umx775umx150um were collected every 30 secs. A 150kDa fluorescently labeled dextran was used to visualize blood vessels and BBB permeability. Images were analyzed using Imaris, Bitplane software, with further processing in Matlab.

Results: Two-photon microscopy demonstrated transient leaks at the pial surface between brain parenchyma and meninges followed by the accumulation of immune cells through the progression of disease. The long term imaging of this disease progression was made possible with the cranial window, two-photon microscopy and fluorescent reporter mice. APC - T cell cell-cell interactions indicate a chemokine directed migration of T cells to the site of inflammation.

Conclusion: We postulate that the leakiness of the blood vessel under inflammatory conditions initiate a cascade of immune events that result in a migration pattern of infiltrating T cells that correlates well with the focal lesion characteristics of multiple sclerosis. Future work includes further defining cellular components in the peri-vascular microenvironment of the BBB which are responsible for regulating the BBB and attracting MOG-specific Th17 cells to cross the vascular barrier into the CNS.

Assessing Margins via Oblique Incidence Diffuse Reflectance Spectroscopy and Confocal Microendoscopy

Jonathan Brand, Dr. Arthur Gmitro, Dr. Bhaskar Banerjee, Dr. Andrew Rouse
University of Arizona College of Optical Sciences

Purpose: Confocal microendoscopy (CME) is a technique to visualize cells and subcellular structures in living tissue. One use of a CME system is to obtain images to assess whether clean margins are achieved following a surgical resection. A clinically important application is evaluation of margins of resected pancreatic tissue in real time at the point of surgery. Oblique Incidence Diffuse Reflectance Spectroscopy (OIDRS) is a technique to measure the absorption and scattering properties of living tissue. OIDRS has the capability to calculate absorption and scattering spectra from larger volumes of tissue than can be imaged with CME. Based on absorption and scattering spectra, OIDRS has the capability to differentiate between healthy and diseased tissue. The hypothesis of this work is that the combination of these two measurement techniques will allow an operator to accurately evaluate surgical margins in real-time in the OR and eliminate the need for the time consuming process of frozen section histology, which is the current standard of care. This approach also has the potential to improve upon this current standard of care by allowing more sites on the tissue to be evaluated than is practical by frozen section histology.

Methods: Tissue from a surgically resected pancreas was obtained from the OR. A site of interest was measured with an OIDRS probe to obtain raw diffuse reflectance spectral data. Acridine orange was then applied to the site as a fluorescent contrast agent. The dyed site was imaged with a separate CME probe. Multiple sites of normal and diseased tissue on each resected pancreas were evaluated Frozen section histology was done on the sites to compare OIDRS and CME results with the gold standard histology results.

Results, Conclusions, and Continued Work: We have successfully collected OIDRS and CME data from pancreatic tissue. Initial analysis shows a difference between OIDRS data and CME images for healthy and diseased tissue. The next phase of the work involves building a combined OIDRS/CME probe to analyze resected pancreatic tissue in the OR. We will quantify the OIDRS analysis with automated pattern recognition methods. A receiver operation characteristic (ROC) study will test the automated pattern recognition algorithms. Expert trained observers will analyze the confocal images and an ROC study will be done comparing their diagnosis to that of gold standard histology. A long term goal is to build a miniature version of the combined probe that can be used in vivo endoscopic procedures.

Three-dimensional oxygen mapping and characterization of stroke pathophysiology

Anthony J. Salvaggio, S.M. Shams Kazmi, Andrew K. Dunn
The University of Texas at Austin

Purpose: Oxygen is one of the vital metabolic molecules required cell survival where a deficit can have a significant physiological impact. During ischemic stroke, delivery of oxygen to the neural network is severely diminished due to a reduction or stoppage of blood flow; this leads to decreased neuronal function as a result of cell death. The goal of this research is to develop the tools necessary to characterize intravascular oxygen transport during ischemia in vivo in order to provide a more comprehensive understanding of stroke pathophysiology.

Methods: To investigate questions regarding intravascular oxygenation requires a combination of tools and techniques including two-photon fluorescence microscopy [31, 32], phosphorescence quenching of oxygen (PQO) lifetime measurements using probe PtP-C343 [35], laser speckle contrast imaging (LSCI) [39], and photothrombosis [41]. Preliminary imaging to identify surface arterioles of interest in the mouse cortex was conducted via LSCI. Two-photon imaging followed to obtain image stacks of the microvasculature, baseline measurements of oxygen tension, and RBC velocities. After baseline measurements, selected occlusion was performed via photothrombosis of targeted vasculature. Specifically, surface arterioles immediately proximal to the area of cortical penetration were targeted. Real-time LSCI monitoring of speckle contrast within the targeted vessel provided feedback on degree and spatial extent of occlusion. Subsequently, two-photon imaging and lifetime measurements were repeated post-occlusion.

Results: Through our work, we have characterized intravascular cortical oxygenation in descending arterioles under baseline and ischemic conditions. Additionally, we have found post-occlusion increases in oxygen tension at branch points along descending arterioles and corresponding changes in flow direction in those branches. Ultimately, evaluation of blood flow and oxygen tension in cortical arterioles will provide important insight into the degree of redundancy present in the vascular network, particularly as primary supply routes are blocked.

Conclusions: With two-photon lifetime microscopy, laser speckle contrast imaging, and photothrombosis, we demonstrate the ability to assess the impact of occlusion on oxygen tension and blood flow, the effect of vascular networking, and the relationship of oxygen with depth in descending arterioles. PtP-C343 has been used to perform highly localized measurements of oxygen tension with two-photon excitation, allowing the creation of three-dimensional maps of intravascular oxygen tension in the cortex under normal and ischemic conditions. This technique will be beneficial to improving our understanding of detailed oxygenation dynamics in three-dimensions with micron resolution.

Super-Resolution Structured Illumination Microscopy of Non-Fluorescent, Coherently Scattering Sample

Joseph Izatt, Al-Hafeez Dhalla
Duke University

Microscopy is critical in the biological sciences for its ability to visualize biological samples at the cellular level. There are many subdivisions under this umbrella of general microscopy, and each are tailored towards specific visualization, design, and contrast requirements. However, a general factor that fundamentally limits the resolution in microscopy is diffraction. However, many biologically interesting structures are at sizes beyond this diffraction limit and are thus irresolvable using typical means. This has prompted many attempts to surpass this diffraction limit to achieve super-resolution. These efforts have found tremendous success in fluorescent imaging, where properties of fluorophores are creatively utilized to visualize the sample beyond the diffraction limit. Such super-resolution techniques generally fall under two broad categories, single molecule detection and point-spread function shaping, and include examples such as stochastic optical reconstruction microscopy (STORM), stimulated emission depletion (STED), and structured illumination microscopy (SIM). However, such super-resolution techniques are limited to fluorescence and typically cannot be applied to super-resolve samples that are highly scattering but NOT fluorescent. Here, we present an extension of conventional SIM to include such samples. We first provide an extensive theoretical framework to describe the mathematics behind the super-resolution reconstruction procedure as well as its fundamental distinction to conventional fluorescent SIM. Next, we set up a proof-of-concept experiment where we demonstrate super-resolution of non-fluorescent structures. We first super-resolve a reflective USA Airforce Test Target and show that the modulation of the spatial frequencies that are resolved in both the widefield and structured illumination setups support the proposed theory and that the super-resolution gain experimentally seen follows theory with excellent matching. After thus characterizing the system and experimentally validating the theory, we show super-resolution of some more typical samples, such as polystyrene beads and histological sides. In all cases, a clear increase in resolution was seen, further pointing to this method's ability to super-resolve real, non-fluorescent, scattering samples.

Direct photoactivation of neurons by laser deposition of thermal energy

Benjamin MIgliori, Massimiliano Di Ventra, William Kristan Jr
University of California, San Diego

Purpose: The ability to precisely activate particular neurons in a living preparation is a crucial tool for understanding neural circuits and behavior. Traditional methods require the use of stimulating electrodes that are manually fixed in a static position during an experiment, which limits the number of neurons that can be driven. This limitation prohibits investigation of many naturally occurring neural activity patterns. We present a method of achieving temporally and spatially precise photoactivation of neurons when genetic expression of photosensitive proteins is unavailable. By replacing electrodes with optical methods, stimulation patterns can be dynamically changed without physically interacting with a target preparation. Such a method would allow investigators to initiate "fictive" behaviors (in which motor patterns are exhibited in the isolated nervous system) via dynamic and physiologically relevant stimuli, and to search for post-synaptic targets in neural circuits.

Methods: Our method depends upon direct conduction of thermal energy via absorption by neutral carbon particles and does not require the presence of voltage-gated channels to create transmembrane currents. We demonstrate photothermal initiation of action potentials in Hirudo verbana neurons and of transmembrane currents in Xenopus oocytes. Thermal energy is delivered by 50 ms, 650 nm laser pulses with total pulse energies between 250 and 3500 μJ.

Results: Our method achieves photoactivation reliably (70 - 90% of attempts) and can issue multiple pulses (3-9) to a given target with minimal changes to cellular properties as measured by intracellular recording. We demonstrate initiation of action potentials in single neurons in the leech ganglion with millisecond precision. We also document an optical delivery system for targeting specific neurons that can be expanded for multiple target sites.

Conclusion: Direct photoactivation presents a significant step towards all-optical analysis of neural circuits in animals such as Hirudo verbana where genetic expression of photosensitive compounds is not feasible. Optical methods can also be expanded to any number of target sites, limited only by available laser power, allowing exploration of complex activity patterns.

Multi-Contrast Optical Coherence Tomography for Neuroimaging

Hui Wang, Adam Black, Thedoen Netoff, Aviva Abosch, Taner Akkin
University of Minnesota

Purpose: Discriminate between white and gray matter by taking advantage of the optical properties of myelinated fibers in rat brain slices.

Methods: Rat brain slices were imaged using a multi-contrast (MC) optical coherence tomography (OCT) system. The system is fiber-based and utilizes polarization maintaining fiber. The light source produces 3.9mW incident power on the sample (brain slice) with the center wavelength of the source at 840nm and the bandwidth at 50nm; this produced an axial resolution of 5.4 microns while the optics of the detection arm resulted in a lateral resolution of 15 microns. The incident light on the sample was circularly-polarized and back scattered light passes through a diffraction grating to split the wavelengths and finally a Wollaston prism to separate the orthogonal polarization states. The light is then detected using a line-scan CCD camera at 20 frames per second. The images acquired contain information on back scattered intensity (reflectivity), phase retardance (birefringence), and relative optical axis orientation. Lastly, to obtain cross-sectional images, the incident light was scanned using a set of galvanometer mirrors producing 1000 A-lines and 250 cross sections resulting in a voxel size of 6.25x25x3.4 um^3.

Results: The slope of reflectivity signal in depth demonstrated a small yet insignificant difference in white matter compared to gray matter. The retardance signals, however, were more pronounced and provided a reliable marker for discrimination between gray and white matter. When the retardance information is combined with relative optical axis orientation, the orientation of the white matter tracts can also be extracted.

Conclusions: MC OCT can provide high resolution three dimensional images that can reliably discriminate between white and gray matter. With tract orientation included, high-resolution images of structural connectivity in the brain are possible when imaged with noninvasive near-infrared light. This imaging system could be useful for obtaining three dimensional connectivity with greater ease than histological images or when histological images are not possible, for example in-vivo applications.

X-Ray

Defining the molecular basis for immune recognition of altered lipid membranes

Gregory Tietjen, Chiu-Hao Chen, James Crooks, Ernesto Vargas, Kathleen Cao, Charles Heffern, Benoit Roux, Binhua Lin, Mark Schlossman, Ka Yee Lee, Erin Adams
University of Chicago

Purpose: The immune system recognizes a vast array of chemical signatures as antigens although historically most research has focused almost exclusively on protein/ protein recognition. More recently it has been appreciated that lipids can also be a potent stimulus for an immune response, as with phosphatidylserine recognition in apoptotic cell clearance. We are studying the molecular mechanisms by which a family of 3 unique phosphatidylserine receptors (Tim family) can directly recognize “out of context” components of the lipid membrane and how this recognition process then stimulates an appropriate immune response. The Tim proteins have been shown to play an integral role in both apoptotic cell recognition and immune regulation. However, despite the demonstrated immunological significance, little is known about the molecular basis for protein/membrane interaction in immune recognition. In large part this is due to the fact that the standard tools of structural immunology, such as x-ray crystallography, are not amenable to protein/lipid interactions. As such we need to develop new combinations of tools capable of addressing basic questions associated with immune related membrane recognition if we hope to understand its fundamental importance.

Methods: We are utilizing x ray surface scattering and advanced modeling techniques, to unveil the structural basis for the recognition of phosphatidylserine containing membranes by each of the three Tim proteins. In addition, Molecular Dynamic simulations have been used to explore the molecular details of recognition.

Results: X ray scattering results have revealed the protein orientation and depth of penetration upon binding to a monolayer mimic of an apoptotic cell membrane. Using this information we have built a high resolution model to address fundamental questions as to which protein residues are involved in binding and how many lipids constitute the 'binding site.'

Conclusions: By combining a variety of tools we can demonstrate that we are able to investigate this protein/membrane system to a resolution sufficient to determine if any functional differences exist between the three PS receptors of the Tim family.

Trauma Imaging with Color Contrast for Color CT: In Vivo Use of Complementary Contrast at DECT

John Mongan, MD, PhD, Samira Rathnayake, Yanjun Fu, PhD, Miguel Cabarrus, MD, Benjamin M. Yeh, MD
University of California, San Francisco

Purpose: We evaluate the ability of a clinical dual-energy computed tomography (DECT) scanner to visually separate pairs of contrast materials selected for maximal differentiability in phantoms and in vivo in a rat cardiac and rabbit abdominal trauma model. We compare accuracy, confidence and speed of diagnosis between conventional CT and dual contrast DECT abdominal trauma imaging. Materials and

Methods: Phantoms with varying concentrations of iodine/tungsten and iodine/bismuth were scanned on a rapid kVp switching DECT scanner. In vivo DECT scans were performed on a rat enhanced with iodinated and tungsten-cluster intravenous contrast agents delivered 15 seconds apart. Five rabbits with bowel and/or vascular penetrating trauma were imaged with simultaneous iodinated intravenous and bismuth subsalicylate enteric contrast at DECT. 10 radiologists without prior DECT experience each evaluated 10 extralumenal collections to record vascular and/or enteric origin of extravasation and their confidence (0 to 100%) in this diagnosis, first with conventional CT images and then with DECT material decomposition density maps. Radiologists also recorded change in perceived speed of diagnosis with addition of DECT images.

Results: Contrast material differentiation was unambiguous both in vitro and in vivo. In the rat, simultaneous pulmonary and systemic arterial phases of contrast enhancement were clearly differentiated. In the rabbit, overall accuracy of identification of source of extravasation increased from 79% with conventional CT to 92% with DECT (157 versus 184 of 200 diagnoses, $p<0.001$). 9 radiologists were more accurate with DECT; 1 had no change. Mean confidence increased from 67% to 81% with DECT ($p<0.001$). In 74% of readings, radiologists found it faster to use DECT than conventional CT alone.

Conclusions: Commercial clinical DECT scanners can distinguish simultaneously administered complementary contrast media in vivo. Further development of complementary contrast media for clinical DECT may significantly improve diagnostic accuracy and confidence in penetrating trauma.

Task-based strategy for optimized contrast enhanced breast imaging in mammography and tomosynthesis

Lynda Ikejimba, Nooshin Kiarashi, Yuan Lin, Baiyu Chen, Sujata Ghate, Moustafa Zerhouni, Ehsan Samei, Joseph Lo
Duke University

Purpose: Currently, mammography is the standard modality in breast cancer screening, despite having a history of low sensitivity and specificity due to tissue superimposition. However, digital breast tomosynthesis (DBT) is a new tomographic modality that produces 3D images of the breast, offering the potential to reduce tissue overlap and improve lesion detection. In addition, the use of an iodine contrast agent in either modality has been shown to improve lesion conspicuity as well as provide functional information through the uptake and washout patterns, information that may help determine if lesion is malignant; often times iodinated image subtraction is necessary to suppress anatomical noise. To determine which imaging protocol may provide the greatest nodule detectability against background, this study analyzes six imaging acquisition and processing paradigms: conventional iodine-enhanced mammography and tomosynthesis, temporal subtraction mammography (TSM) and tomosynthesis (TST), and dual energy subtraction mammography (DEM) and tomosynthesis (DET). To our knowledge, this is the first study to perform a rigorous quantitative analysis of these imaging techniques.

Methods: The study developed a Fourier-based figure of merit called a detectability index, or d', to quantitatively validate each technique. The index is defined as a function of the following parameters: k is the spatial frequency; TTF is the task transfer function, a measure of how well the original spatial information is preserved; S is the function describing the shape of the lesion, and FT{S} is its Fourier transform; C is the iodine contrast, and NNPS is the normalized noise power spectrum, which includes anatomical and quantum noise as a function of dose. All parameters were obtained using a prototype Siemens MAMMOMAT Inspiration system in mammography and tomosynthesis modes, except for S, which was mathematically developed. The TTF was measured using an acrylic and iodine-doped edge, from which an edge spread function was differentiated and Fourier transformed to produce the TTF. The contrast values were acquired using breast equivalent materials doped with varying amounts of iodine concentration, ranging from 2.1 mg/cc to 8.6 mg/cc. Lastly, the NNPS spectra calculated using a CIRS model 020 phantom.

Results: For unsubtracted images, tomosynthesis produced higher results. However, subtraction techniques generally yielded higher d' values for both modalities. In particular TST had the highest d' overall, followed by DET and DEM.

Conclusions: For images obtained with the lowest dose and lowest iodine concentration, tomosynthesis and image subtraction techniques are optimal compared to conventional iodine-enhanced mammography.

Lung Texture in Serial Thoracic CT Scans: Assessment of Change Introducted by Image Registration

Alexandra R Cunliffe, Hania Al-Hallaq, Ph.D., Zacariah E Labby, Ph.D., Charles Pelizzari, Ph.D., Christopher Straus, M.D., William Sensakovic, Ph.D., Michelle Ludwig, Samuel G Armato III, Ph.D.

University of Chicago, Medical Physics

Purpose: To quantify the effect of four image registration methods on lung texture features extracted from serial computed tomography (CT) scans.

Methods: Two chest CT scans acquired at different time points were collected retrospectively for each of 27 patients. Following automated lung segmentation, each follow-up CT scan was registered to the baseline scan using four algorithms: (1) rigid, (2) affine, (3) B-splines deformable, and (4) demons deformable. The registration accuracy for each scan pair was evaluated by measuring the Euclidean distance between 150 identified landmarks. Over 1,600 spatially matched 32x32-pixel region-of-interest (ROI) pairs were automatically extracted from each scan pair. First-order, fractal, Fourier, Laws' filter, and gray-level co-occurrence matrix (GLCM) texture features (140 features total) were calculated in each ROI. Agreement between baseline and follow-up scan feature values was assessed by Bland-Altman analysis for each feature; the range spanned by the 95% limits of agreement was calculated and normalized by the average feature value to obtain the normalized Range of Agreement (nRoA). Features with small nRoA were considered "registration-stable." The normalized bias for each feature was calculated from the average feature value differences between baseline and follow-up scan ROIs. Because patients had "normal" chest CT scans, minimal change in texture feature values between scan pairs was anticipated, with the expectation of small bias and narrow limits of agreement.

Results: Following demons registration, only 9% of landmarks were separated by a Euclidean distance = 1mm, compared with rigid (98%), affine (95%), and B-splines (90%). 99 of the 140 (71%) features analyzed yielded nRoA > 50% for all registration methods, indicating that the majority of feature values were perturbed following registration. Nineteen of the features (14%) had nRoA < 15% following demons registration, indicating relatively feature value stability. For 16 of these 19 features, nRoA was larger when rigid, affine, or B-splines registration were used compared with demons registration. The remaining three features yielded similar nRoA for all registration methods. Demons registration affected the baseline value of features to a greater extent (|Bias| > 1% for 15 of the 19 features) than B-splines registration (|Bias| > 1% for seven of the 19 features).

Conclusion: There is a subset of texture features that remain relatively stable following demons deformable registration of serial CT scans. Combined use of demons registration and texture analysis may allow for quantitative evaluation of local changes in lung tissue due to disease progression or treatment response.

Bariatric Arterial Embolization with X-ray-visible embolic beads and c-arm cone beam CT for increase

Paul Allen DiCamillo, Weijie Beh, Charles Hu, Tza-Huei Wang, Hai-Quan Mao, Dara L. Kraitchman, Clifford R. Weiss
Johns Hopkins University, Dept. of Radiology

Purpose: Ghrelin, a potent endogenous hunger stimulant, has been a target for weight loss. Ghrelin levels may be manipulated by embolizing its production centers in the gastric fundus; however, incomplete fundal embolization and concerns for non-target embolization remain obstacles to success. We employed X-ray visible embolic beads (XEBs) and c-arm Cone Beam Computed Tomography (CBCT) to increase the accuracy of embolizing the arterial supply to the gastric fundus, and confirmed our work histopathologically.

Materials and Methods: Barium sulfate containing, highly uniform alginate XEBs (~50 μm) were custom made as the embolic agent in this study. Targeting of the arteries supplying the gastric fundus in 3 swine was determined using CBCT [dynaCT, AXIOM Artis dFA (Siemens Healthcare, Forchheim, Germany) or Philips Allura Xper (Philips Healthcare, Andover, Massachusetts, USA)], 8s DSA, 210° rotation, 0.5°/step] as follows. Employing a femoral approach, the celiac axis was selected with a 5F Mickelson catheter. After angiogram, each of the 3-4 fundal arteries was selected using a 0.016 fathom wire with a Renegade Hi-Flow catheter. At each target location a pre-embolization angiogram and CBCT (25-50% contrast, 1 cc/sec for 10 sec) were performed, the artery was embolized with XEB's under direct fluoroscopic visualization, and a non-contrast postembolism CBCT was completed. Prior to sacrifice, a black tissue dye was injected from the celiac artery to confirm post-embolic circulation. H&E sections of the stomach were prepared and evaluated.

Results: We mapped the vasculature and selectively embolized the gastric tree of three swine, and confirmed the precise targeting to the fundal submucosa histopathologically. The XEBs were visible during both fluoroscopic delivery and CBCT in all studies, reflux and non-target injection was visualized at time of delivery, and embolic coverage was confirmed intra-operatively via CBCT.

Conclusions: We confirmed histopathologically that the combination of XEBs and CBCT facilitated accurate mapping of the gastric fundus and enhanced safety by reducing the risk of back flow, thus sparing non-target tissue embolization. Further study will be necessary to evaluate bariatric arterial embolization as a therapy for morbid obesity.

Objective Evaluation of CT Image Reconstruction Algorithms

Adrian Sanchez, Emil Sidky, Xiaochuan Pan
University of Chicago

Purpose: Objective image evaluation through mathematical model observers provides a systematic means for ascertaining signal detectability in a two state classification task such as the detection of cancerous lesions in an image obtained through x-ray computed tomography (CT). In particular, model observer metrics such as the Hotelling template or the Hotelling SNR can provide information regarding the loss of signal detectability through the steps of a linear CT image reconstruction algorithm. Moreover, the implementation of CT image reconstruction algorithms (the mapping of projection data into a final CT image) requires many choices to be made. Herein, we employ the Hotelling template as a tool for evaluation of various implementation choices in a single analytic reconstruction algorithm: the back-projection filtration (BPF) algorithm developed by our group.

Methods: The noise model in the projection domain was taken to be independent, identically distributed (IID) Gaussian noise of zero mean and unit variance. The particular implementation choice investigated was the implementation of the numerical directional derivative of the projection data. Forward differencing was investigated alongside discrete Fourier transform (DFT) derivatives of various kernels.

Results: Even for implementation changes to which the Hotelling SNR (a summary scalar metric) was insensitive, we saw significant changes in the structure of the Hotelling template, the image with which the signal is masked to make the signal absent/signal present decision. In particular, we hypothesize that trivial prewhitening tasks (those with a template that resembles the signal) will correlate with improved human detection of signals in CT images. The linear kernel with DFT derivative implementation was found to have the template most resembling the signal, and is therefore hypothesized to be the implementation most conducive to human signal detection, of the options considered here.

Conclusions: The Hotelling observer, and the Hotelling template in particular, can provide valuable information regarding the detectability of signals in a reconstructed CT image, as well as the extent to which an observer must perform prewhitening in order to detect those signals. We therefore conclude that the Hotelling observer, as well as other mathematical model observers, show promise for aiding in CT image reconstruction algorithm design through ensuring optimality of implementation with regard to signal detectability.

Neutron Dosimetry in a Voxelized Anthropomorphic Phantom Using Monte Carlo Methods

Matthew Belley, Anuj Kapadia
Duke University, Medical Physics

Purpose: In this study, we estimated the 3-dimensional dose distribution for a Neutron Stimulated Emission Computed Tomography (NSECT) scan of the liver using the GEANT4 Application for Tomographic Emission (GATE) Monte-Carlo toolkit and the XCAT human phantom. NSECT is a technique that facilitates in-vivo imaging of the spectroscopic distribution of elements in the body using fast neutrons by measuring and quantifying the relative amounts of emitted characteristic gamma rays from the (n, n', gamma) reaction. This technology is being developed to detect changes in elemental concentration that can be early indications of diseases such as breast cancer and iron overload in the liver. Understanding the 3-dimensional dose distribution from such a diagnostic scan is essential for assessing the radiation risk to patients.

Methods: The XCAT phantom, a whole body human computer model, was used as the input to GATE as a spatial map of the material composition of the body. A male model of the torso was voxelized into 256x256x100 voxels of size 3.125x3.125x3.125 cu. mm. For the NSECT scan, a 5 MeV monoenergetic neutron pencil-beam source with a fluence of 1 million neutrons per sq. cm was simulated to scan a 2 cm thick slice in the axial direction at eight different angles in increments of 45 degrees around the torso.

Results: Without applying tissue-weighting factors the maximum dose in the volume, which occurred at the edges of the torso at the beam entrance, was found to be 0.0032 cGy. The dose was found to decrease along the direction of the beam, reaching a minimum value of about 0.0010 cGy in the soft tissue at the center of the phantom (a nearly three-fold reduction). The dose values measured in regions such as the ribs and spine (with relatively lower hydrogen content) showed a sharp reduction compared to the neighboring hydrogen-rich tissue. A 42% reduction in hydrogen density in the rib bone compared to the surrounding body correlated to a decrease in the dose of about 55-60%. In the axial direction, the maximum dose was found to be centered on the beam plane, dropping to about 10% of the maximum dose at 2.5-3 cm from the edge of the beam plane.

Conclusion: The results indicate that neutron-illumination of organs such as the liver imparts non-uniform dose to the primary organ with negligible dose delivered to neighboring proximal organs outside of the beam plane.

Nuclear Medicine

Quantification of Tc-99m Sestamibi in asymptomatic breast tissue using dedicated breast SPECT-CT

Steve D Mann, Kristy L Perez, Jainil P Shah, Martin P Tornai
Duke University

Purpose: To quantify Tc-99m Sestamibi (MIBI) in normal, asymptomatic breast tissue for evaluating the potential of a global threshold for cancer diagnosis or staging. Women with normal risk of developing breast cancer were imaged using a dedicated breast SPECT-CT system, and the resultant data was quantified to determine the average baseline uptake of MIBI.

Methods: Seven female subjects undergoing diagnostic parathyroid MIBI studies were consented and imaged as part of an IRB-approved pilot study for breast MIBI quantification. Subjects were imaged with our dedicated breast SPECT-CT system between their routine scintigraphy (10min post 25mCi injection) and SPECT (2hrs post injection) diagnostic parathyroid scans. Women weighing >160kg, possible pregnancy, or with any breast cancer history were excluded. The CT system has a quasi-monochromatic x-ray beam (mean energy is 36 keV) and a 25x20cm^2 flatpanel detector. The orthogonally mounted SPECT system utilizes a 16x20cm^2 CZT gamma camera capable of acquiring projections using non-traditional trajectories. Subjects lay prone on a custom bed with their breast hanging pendant into the field-of-view of the system. SPECT scans were performed using contoured, sinusoidal 3D trajectories. All SPECT data was scatter and attenuation corrected using previously established techniques, reconstructed using OSEM, and the results were decay corrected to the time of injection. CT data sets were reconstructed using OSC and segmented using a threshold technique to separate glandular and fatty tissue. Resultant SPECT and CT images were registered, and the average activity concentrations of fatty, glandular, and the whole breast tissue were measured.

Results: Results show a mean whole breast activity concentration of 0.09uCi/mL. In contrast to FDG-PET breast imaging, no significant differences between glandular and fatty tissue uptake were discernible. The pilot study indicates that approximately 30 subjects are necessary for a 95% confidence for whole breast MIBI quantification.

Conclusion: Preliminary results show no differences in MIBI uptake for different breast tissue types, indicating that a global threshold may be useful for staging or diagnosing malignant tissue. Additional subjects are needed to increase the confidence in the measured values.

Automated 18F Labeling of Sucrose for Transporter Studies in Plants via PET

Tom Brossard, David Rotsch, Vikram Gaddam, Michael Harmata, J. David Robertson, David Braun
University of Missouri

Purpose: The project objective is to fluorinate sucrose with 18F at the 5 position of the fructosyl unit of the molecule with an automated radiochemical process. Once radiolabeled, the tracer will be used to map the uptake and transport of sucrose by sucrose transporters, specifically SUT1, in SUT1 deficient mutants and wild-type maize (Zea mays) leaves via positron emission tomography (PET).

Methods: Fluorine-18 will be produced with a GE PETtrace by the 18O(p,n)18F reaction on a heavy water target. Following transfer of the 18F, to the automated chemistry system in the shielded cell, the fluorine is dried twice with acetonitrile and then transferred to the reaction cell. 6'-O-trifluoromethanesulfonyl-2,3,4,6,1',3',4'-hepta-O-benzoylsucrose (triflated sucrose) and Kryptofix 222 are then added and refluxed for 10 minutes under argon. Next, potassium carbonate and methanol are added and refluxed for another 5 minutes to quench the reaction. The product is separated and purified by HPLC.

Results: A cold run using potassium fluoride in place of 18F from the cyclotron to selectively fluorinate the triflated sucrose was recently completed. The product was characterized using 19F and proton NMR confirming the synthesis of the fluorinated sucrose analogue.

Conclusion: We have synthesized fluorinated sucrose with cold materials with an automated chemical synthesis system in the shielded cell and will attempt to label sucrose with 18F in the coming weeks. The radiolabeled compound will be used to map the HPLC elution profile of the product. Previously, using 14C labeled sucrose on an autoradiograph, the uptake of sucrose by SUT1 was demonstrated while a lessened transport of sucrose was observed in the mutant plant.1 Using the 18F labeled sucrose, we will observe the transport of sucrose in both the mutant and wild-type plants in real-time. We will also use the tracer to investigate where the sucrose is locally stored in the maize plant.

1 Slewinski, T.L., Meeley, R., Braun, D.M.; "Sucrose transporter1 functions in phloem loading in maize leaves", Journal of Experimental Botany, 60(3), 2009, pp. 881-892.

Development and Quantification of a Novel Intravascular Catheter-Based Radionuclide Imaging System

RT. Zaman, CM.Carpenter, G. Pratx, C. Sun, L. Xing, MV. McConnell
Stanford University School of Medicine

Objectives: Atherosclerosis is a progressive inflammatory condition that underlies coronary artery disease (CAD)—the leading cause of death in the United States. In this study, we developed a novel intravascular catheter-based optical system to image FDG, a marker of vascular inflammation, and characterized the system for spatial resolution and signal intensity from various radiation sources.

Methods: The catheter system included a filter wheel placed between 35 mm and 8 mm fixed focal length lenses, which were subsequently connected to a cooled EM CCD and fiber holder. The proximal end of a leached image bundle was attached to the fiber holder and the distal ferrule was terminated with a wide-angle lens. To convert the decay of FDG signal into light, we fabricated a scintillating membrane from 1mL of silicone RTV catalyst mixed with 1 mL base. YAG (0, 5, 50 mg/mL) or anthracene (0, 2.5, 25 mg/mL) phosphors were added to the silicone; three different thicknesses were measured at each concentration. To identify the optimal scintillating membrane with respect to brightness, the scintillator was placed over Co-57 (1.25 µCi) and Tl-204 (0.1µCi) radiation sources and signal intensity was appropriately corrected for background and flat-field. To identify the optimal scintillating membrane with respect to resolution, we calculated MTF using a thin line optical phantom.

Results: Analysis of the different radiation sources and phosphors showed that anthracene (204 a.u.) was 60% brighter compared to YAG (81.4 a.u.). Also, a similar concentration of anthracene enabled 2.5µm resolution compared to YAG (6.7µm) and overall system resolution was 20µm.

Conclusions: Anthracene provided better sensitivity and spatial resolution as a scintillating membrane for this novel catheter-based system. With further implementation, we expect this to be a potentially useful tool in evaluating FDG uptake in coronary atherosclerosis.

Research Support: This work was supported by a training grant from the National Institutes of Health (T32EB009035).

72,77As labeled radiopharmaceuticals for use as diagnostic imaging and/or therapeutic agents

Anthony J. DeGraffenreid, Don Wycoff, Ryan Morrow, Beau Ballard, Cathy S. Cutler, Alan R. Ketring, Michael Fassbender, Silvia S. Jurisson
University of Missouri - Columbia

Efforts to yield suitable imaging and therapeutic isotopes of arsenic are being explored at Los Alamos National Laboratory (LANL) and the University of Missouri's research reactor (MURR). 72As is available from the decay of 72Se (t1/2 8.4 d, EC) has been produced from near spallation 100 MeV protons, on a NaBr target, at the LANL accelerator. Alternatively, 77As is available at MURR through neutron bombardment of 76Ge. Arsenic has the potential to be used for PET imaging (72As: t1/2 26 h, ß+ 2.49 MeV) and radiotherapeutic applications (77As: t1/2 38.8 h, ß- 0.683 MeV). If these radionuclides are to be widely used in radiopharmaceutical applications an efficient route to produce, separate, and synthesize stable complexes is necessary. Utilization of these radioisotopes as radiopharmaceuticals requires the development of compounds with high in vivo stability that can be conjugated to targeting probes. To this end, the reaction of p-arsanilic acid and phenyl arsonic acid with various dithiols was investigated. The compounds were analyzed by 1H and 13C NMR, electrospray ionization mass spectrometry (ESI-MS), and single crystal X-ray diffraction.

Ultrasound

Evaluation of AVUS treatment effects by DCE-US, DCE-MR, and histopathology.

Stephen Hunt, MD, PhD, Andrew K Wood, DVM, Michael Soulen, MD, Terence Gade, MD, PhD, Steve Pickup, PhD, Chandra Sehgal, PhD
University of Pennsylvania

Purpose: To study the frequency and temporal-dependency and treatment effects of anti-vascular ultrasound (AVUS) with dynamic contrast-enhanced magnetic resonance (DCE-MR) and ultrasound (DCE-US) imaging in a murine melanoma model and validate the imaging results with histopathology.

Methods: Subcutaneous K1735 murine melanoma tumors were grown in 30 syngeneic C3H/HeN mice to approximately 1.5 cm maximal diameter. Quantitative tumor perfusion characteristics were measured immediately prior to treatment with both high-resolution DCE-US and DCE-MR at 9.4 tesla. Tumors were subsequently treated with 1 or 3 minutes of continuous low-intensity ultrasound (2.3 W/cm2) at 3 MHz frequency after intravenous administration of Definity ultrasound contrast. Controls received 3 minutes sonication with identical sonication parameters in the absence of Definity. Post-treatment antivascular effects were subsequently assessed by quantitative DCE-US, DCE-MR, and histopathology.

Results: Low-intensity AVUS treatment results in a potent reduction in tumor perfusion as assessed by imaging. One minute of AVUS treatment resulted in approximately a 40% reduction in tumor perfusion on post-treatment DCE-US, while 3 minutes resulted in approximately 70% reduction in tumor perfusion on post-treatment DCE-US. DCE-MR measurements demonstrated corresponding changes in tumor perfusion parameters, with a doubling and quadrupling of time to peak enhancement in the 1-minute and 3-minute treatment groups respectively. The magnitude of gross pathology and histologic changes correlated with the treatment time, and qualitatively with the regions of perfusion defect seen on DCE-US and DCE-MR. The predominant histopathologic findings seen at 24-hours were dilatation of tumor capillaries, thrombosis, intra-tumoral edema, perivascular hemorrhage, and inflammatory infiltration. Sonication in the absence of microbubbles did not demonstrate sustained measurable effects on tumor perfusion kinetics or histopathology.

Conclusions: Contrast-enhanced ultrasound holds great promise for both diagnostic and therapeutic applications in oncology. AVUS treatment has a potent antivascular effect, with histopathologic changes including vascular thrombosis and intra-tumoral hemorrhage. In addition to resulting tumor ischemia, preliminary data suggests a possible role for the AVUS technique in increasing targeted chemotherapeutic delivery and endogenous vaccination. Lack of contrast microbubbles eliminates the treatment effects, demonstrating the importance of microbubbles in generating the local therapeutic effects.

Full-Field Transmission Ultrasound Imaging System Employing an Acouto-Optic (AO) Detector

J.R. Rosenfield, J.S. Sandhu, J.K. Tawiah, P.J. La Riviere
The University of Chicago, Dept. of Radiology

Purpose: In this work, the spatial resolution and noise properties of a prototype full-field transmission ultrasound imaging system employing an acousto-optic (AO) liquid crystal detector were characterized. The AO effect is a phenomenon in which an incident acoustic wave field induces local birefringence changes in a liquid crystal. These birefringence changes manifest as brightness changes when the liquid crystal is optically illuminated using polarized light, thus providing spatial information about the field.

Methods: A compressed, Zerdine-based breast phantom containing 12 artificial spherical lesions was imaged using plane-wave ultrasound illumination. Lesions of diameter 2 mm, 4 mm, 6 mm, and 8 mm were embedded at depths of 12.7 mm, 25.4 mm, 38.1 mm within the phantom background. To minimize coherence artifacts, the transducer frequency was swept continuously from 3.25 MHz to 3.45 MHz at a rate of 100 MHz/s. The transducer voltage was ramped from 0.1 V to 6.5 V to permit identification of the onset of the AO effect in the detector. An analysis of image quality was performed on 50 identically acquired images in which the contrast-to-noise ratio was determined for each lesion in the mean acquired image. Apparent lesion size was also computed as a function of distance from the AO detector.

Results: The spatial resolution analysis revealed that lesion size in the mean acquired image increased linearly with lesion-to-detector distance. Extrapolation of the least squares regression lines for apparent lesion size versus lesion-to-detector distance to zero distance agreed well with the actual lesion sizes. The noise analysis demonstrated that a contrast-to-noise ratio of 13.1 could be obtained with the prototype system for the transducer settings and phantom properties considered.

Conclusions: This investigation indicates the potential for incorporating a liquid crystal AO detector into a transmission ultrasound system for full-field breast imaging.

Towards imaging inflammation with ultrasound based molecular imaging.

Olson, Emilia, Wu, Clark, Yi, Boemha, Gao, Wei, Eghtedari, Mohammad, Orozco-Holguin, Jahir, Wang, Joseph
University of California, San Diego

Purpose: We present a novel ultrasound (US) molecular imaging approach that aims to produce microbubbles (MB) in situ. These US agents aim to catalyze hydrogen peroxide (H2O2) one of the common reactive oxygen (O2) species in vivo, into water and O2 MBs to act as US contrast agents. Since H2O2 is produced in 60-100 µM quantities by activated neutrophils in inflammatory tissues, our first in vivo application will be to detect infections. Micromotors (2x10 µm cones) have been designed to move through fluids by catalyzing H2O2 to produce and release O2 MBs as propellants; the higher the H2O2 concentration the more O2 MBs are produced and the greater the velocity. Minimal detectable motion required =65mM of H2O2, ~1000x the expected in vivo concentration. In this study we aimed to demonstrate the feasibility of detecting the produced O2 MBs with US, determine the minimum H2O2 required for MB detection, and begin to optimize the design as we progress towards in vivo testing.

Methods: 8000 micromotors of standard design or optimized by coating the interior wall with catalase to increase the rate H2O2 catalysis were placed in a transfer pipette along with 0.04M sodium cholate hydrate as surfactant and PBS and imaged with B-mode and contrast specific modes. H2O2 was added incrementally in small quantities to each suspension to determine the minimum H2O2 concentration that produces US detectable MBs.

Results: Generated O2 MBs were visible with both US imaging techniques. The minimum H2O2 concentration needed for US detection with the standard micromotors was 2,500 µM and with the catalase-coated micromotors 300 µM.

Conclusion: We were able to detect the O2 MBs produced by the micromotors when they catalyzed H2O2, and were able to decrease the minimum H2O2 concentration required for US detection by nearly an order of magnitude by coating the inner lumen with catalase. The current detection limit approaches in vivo H2O2 concentrations. Future experiments will continue to improve upon the current design to further decrease the H2O2 detection limit and image a suspension of activated neutrophils to provide proof-of-concept that such an approach can be used to detect areas of inflammation in vivo.

Application of Synthetic Aperture Focusing to Short-Lag Spatial Coherence Imaging

Nick Bottenus, Dongwoon Hyun, Jeremy Dahl, Gregg Trahey, Brett Byram
Duke University

Spatial coherence describes the similarity of a returned echo signal across the aperture of an ultrasound array as a function of distance, or lag. Short-lag spatial coherence (SLSC) imaging forms an ultrasonic image by integrating this coherence curve across several lags. Application of the Van Cittert Zernike (VCZ) theorem predicts that spatial coherence falls off away from the transmit focus, limiting the depth of field (DOF) in the SLSC image. Synthetic aperture (SA) focusing uses received channel data from multiple transmit events to create beams that are continuously focused on transmit and receive. We apply SA focusing to improve spatial coherence away from the transmit focus and improve DOF in SLSC imaging. Individual channel data are collected using a linear array transducer and a conventional focused transmit. A scan comprising 128 transmits was collected from a phantom containing anechoic and hypoechoic lesions using 80 elements for transmit and 128 elements in receive. The focus was placed 30.8 mm from the transducer with shallow lesions at 40 mm and a second anechoic lesion at 70 mm. The anechoic lesions were placed at the edge of the field of view due to physical constraints. Synthetic aperture techniques were used to sum contributions from virtual sources in the field to create a larger channel and transmit count focused at each point. SLSC imaging techniques were applied to the SA focused signals to generate SLSC images with an extended depth of field. We show a comparison between conventional and synthetic SLSC images. The CNR of the anechoic lesion at 40 mm improved with SA focusing from 4.7 to 11.0 and SNR improved from 8.6 to 12.8. The SA focused SLSC image shows improved SNR and CNR through depth, evidenced by the anechoic lesion at 70 mm. The DOF increased from 23.0 mm to 58.8 mm and shows better uniformity in brightness and resolution. The spatial coherence functions confirm the predictions of the VCZ theorem, showing a curve that falls off non-linearly for the dynamic receive case in a homogeneous region outside the transmit focus. Synthetic aperture methods improve coherence, showing a linearly decreasing curve. This project was supported by grants from the NIBIB (R01-EB013661, T32-EB001040).

Ultrasound Contrast Agents to Enhance Localized Drug Delivery in Cancer

Anna Sorace, Reshu Saini, Jason Warram, Heidi Umphrey, Kurt Zinn, Kenneth Hoyt
University of Alabama at Birmingham

Purpose: Ultrasound contrast agents, or microbubbles, have recently emerged in the cancer field as a novel technique for enhancing detection, monitoring and delivery of therapeutics. Ultrasound exposed microbubbles have the capability to transiently enhance localized molecular delivery through mechanical oscillations. Using this technique in cancer treatment through localized delivery can increase overall efficiency of chemotherapeutic drug delivery to the target tumor. This study optimizes ultrasound parameters for maximum drug delivery and utilizes other imaging strategies to evaluate the logistics of microbubble-mediated ultrasound therapy.

Methods: Using a single element immersion transducer in series with a power amplifier and signal generator, microbubble-mediated ultrasound therapy parameters were investigated. The influences of ultrasound parameters such as pulse repetition period, mechanical index, transmit frequency and duration of exposure can affect the reactions of therapy. Microbubble-mediated ultrasound therapy can be explored both in vitro and in vivo for increased molecular uptake. Using flow cytometry, in vitro quantification of increased fluorescent uptake of a membrane impermeable molecule in breast cancer cells was investigated. This technique was optimized using fluorescent molecules, then implemented and analyzed with combination chemotherapy plus microbubble-mediated ultrasound therapy. 2LMP breast cancer cells were implanted subcutaneously in nude athymic mice (N = 42) and tested for increased drug delivery of paclitaxel using microbubble-mediated ultrasound therapy. An additional study used fluorescent molecules in place of chemotherapy (Cy5.5 dye) and optical imaging (Pearl Impulse small animal optical imaging system) to further analyze temporal effects of microbubble-mediated therapy in a breast cancer animal model (N=12). Region-of-interest analysis in the tumor analyzed fluorescent uptake. ANOVA statistical analysis was conducted (SAS software).

Results: Combination chemotherapy and microbubble-mediated ultrasound therapy with optimized parameters increased cancer cell death by 50% over chemotherapy alone ($p = 0.003$). Breast cancer tumor bearing mice were analyzed for increased therapeutic effects of microbubble-mediated ultrasound therapy plus chemotherapy, showing a 42% decrease in tumor size compare to drug alone when using a mechanical index of 0.5 ($p = 0.037$). Optical imaging resulted in an 18% increase of fluorescent dye delivery compared to control counterparts ($p = 0.02$).

Conclusion: Optimized microbubble-mediated ultrasound therapy in breast cancer has potential to improve patient response to therapy via increased localized drug delivery. This may lower chemotherapeutic drug dosages, thereby reducing systemic toxicity and improving patient response. Future studies include targeting the MBs to receptors that are overexpressed in cancer and evaluate targeted and localized drug response to microbubble-mediated ultrasound therapy.

Imaging Agents & Molecular Probes

Cathepsin K Radioligands for In Vivo Imaging

Melissa E. Topper, Xia Shao, Peter J. H. Scott, Michael R. Kilbourn
University of Michigan

Osteoporosis is the most prevalent form of bone disease, and is defined by low bone mass and microarchitectural deterioration of bone tissue, resulting in bone fragility and vulnerability to fractures. An estimated 1 in 4 men and 1 in 2 women over the age of 50 will suffer from an osteoporosis-related fracture in his or her lifetime. In 2005, the direct costs associated with osteoporosis were estimated to be $19 billion dollars. Excessive activity of cathepsin K, a cysteine peptidase whose primary location is in the osteoclast, has been linked to osteoporosis. Recently reported literature demonstrates the utility of cathepsin K as a biomarker for identifying indirect in vivo osteoclast upregulation prior to detectable bone loss. Additionally, there has been successful fluorescence in vivo imaging of cathepsin K and osteoclasts in animal models. The purpose of this project involves the development of novel imaging agents of cathepsin K that will act as prognostic indicators of increased osteoclast upregulation prior to significant detectable bone loss, providing immediate feedback on the efficacy of a treatment plan and allowing for earlier diagnosis of the disease. The methods used to achieve this purpose includes the design, synthesis, and biological evaluation of novel radiotracers for positron emission tomography (PET) imaging of musculoskeletal diseases involving aberrant osteoclast activity, namely osteoporosis. More specifically, this project will focus on the synthesis of radiolabeled forms of selected potent cathepsin K inhibitors for PET imaging, and assessment of their in vivo osteoclast-specific localization. The projects current results include the complete synthesis and characterization of two distinctive potent inhibitors of cathepsin K based on a cyano-pyrimidine scaffold (IC50 values of 0.022 and 0.003 nM, respectively). The target molecules each maintained an aryl methoxy substituent that was suitable for isotopic labeling with carbon-11. Using an automated radiosynthesis platform, the syntheses of the carbon-11 labeled radiotracers were accomplished by reaction of the O-desmethyl precursors with no-carrier-added [C-11]methyl iodide. In conclusion, the carbon-11 labeled radiotracers are to date the first available cathepsin K inhibitors that have been labeled with a positron emitting radionuclide for potential in vivo imaging use. The radiotracers are currently being evaluated in normal rodents for whole-body biodistribution, metabolism, and route of clearance.

In Vivo Bacteriophage Display Selection for an Improved Breast Cancer Targeting Peptide

Benjamin Larimer, Susan Deutscher
University of Missouri

Purpose: Each year more than 200,000 women are diagnosed with breast cancer and 40,000 women die from the disease. Current detection methods, including breast exams and mammograms, are essential tools for diagnosing large carcinomas; however both are ineffective at diagnosing smaller, rapidly dividing malignancies. To supplement traditional imaging methods, considerable effort has been invested in devising molecular techniques to image cancer at the cellular level. Molecular imaging relies on cancer-specific antigens, such as the epidermal growth factor receptor, and ErbB-2. ErbB-2 is expressed on a majority of breast cancers and overexpressed on up to 30 percent. Although monoclonal antibodies are traditionally used to target cancer biomarkers, including ErbB-2, peptides may offer a preferential alternative due to their improved pharmacokinetic properties. Bacteriophage (phage) display is a technique utilizing a sample population of up to a billion unique peptides to screen for a high affinity targeting molecule. We believe that phage display can also be used to affinity maturate a known ErbB-2 targeting peptide, KCCYSL, to improve the in vivo biodistribution of a novel peptide containing the original binding sequence.

Methods: Filamentous phage were engineered to bear the original ErbB-2 targeting peptide sequence, KCCYSL, flanked by nine random amino acids. The new "KCCYSL Library" was subjected to several rounds of in vivo selection in mice bearing human breast tumor xenografts. The tumor avid phage were isolated and verified for breast cancer specific affinity. The displayed peptides of the four clones possessing highest affinity for human breast carcinoma cells were chemically synthesized to assess binding. Fluorescent and radiolabeled peptide assays were used to confirm specific binding of the peptides. One radiolabeled peptide was selected for in vivo analysis of pharmacokinetics.

Results: The in vivo phage selection resulted in numerous unique phage candidates. The displayed peptides of the top four phage were synthesized and tested in vitro. Fluorescence based assays revealed two peptides with high affinity and specificity. Once radiolabeled, only one peptide, 1-D03, demonstrated affinity and specificity for breast cancer cells. Pharmacokinetic analysis in mice bearing human breast tumor xenografts revealed a 20 percent decrease in kidney retention, and SPECT/CT imaging confirmed tumor detection.

Conclusions: In vivo phage display is a viable technique for enhancing the pharmacokinetic properties of a breast tumor targeting peptide. Further characterization of the contributions of individual amino acid to tumor affinity and biodistribution may allow for enhanced tumor uptake and a further reduction in non-target accumulation.

Quantitative susceptibility mapping calculation dependence on contrast agent and field strength

Russell Dibb, Wei Li, Chunlei Liu
Duke University

Purpose: Quantitative susceptibility mapping (QSM) produces MRI contrast that directly reflects the magnetic properties of different biological substrates. For instance, loss of diamagnetic myelin in the central nervous system can be visualized using QSM. Magnetic susceptibility contrast, however, depends strongly on the presence of paramagnetic contrast agents and the magnetic field strength applied during the scan. Characterizing these dependent relationships would aid in the effective use, comparability, and consistency of QSM.

Methods: Six C57BL/6 mice were perfused first with a mixture of 0.9% saline and one of six different concentrations of ProHance (0-10%) (Bracco Diagnostics, Princeton, NJ), followed by a mixture of 10% buffered formalin (Buffered Formalde-Fresh; Fisher Scientific). The skulls were removed from the body with the brain intact. Data for each brain specimen were acquired using a gradient-echo sequence (TE = 5.0ms, TR/FA/BW = 500ms/90°/62.5kHz, 86x86x86 μm voxels) at field strengths of 2, 7, and 9.4 Tesla. Four excitations were acquired at 2T to achieve adequate SNR. In order to calculate the susceptibility maps, the signal phase data underwent unwrapping, background phase removal, and deconvolution operations to invert the equation relating magnetic susceptibility and frequency offset. The data were then normalized according to the applied magnetic field. It is important to note here that the frequency offset and magnetic susceptibility are referenced to the carrier frequency of the excitation RF pulses set during the pre-scan—making both values relative measures. However, using adjacent gray matter as an internal reference allows for useful comparisons to be made between datasets.

Results: For each dataset, the mean susceptibility of the voxels comprising the white matter of the anterior commissure relative to the gray matter reference were plotted against the independent variable, contrast agent concentration. A linear regression model, was fit to the data in the three white matter regions examined in this study. The variation seen among white matter regions is likely due, in part, to the angle of the fiber tracts with respect to the applied field and the anisotropic magnetic susceptibility properties of myelin.

Conclusions: As predicted by the theoretical model, the experimental data illustrate that white/gray matter contrast increases linearly with increasing field strength. This contrast also increases linearly as the concentration of ProHance in the specimen increases, i.e. the introduction of a paramagnetic contrast agent into the specimen results in the myelinated white matter appearing to be relatively more diamagnetic, while gray matter appears to be largely unaffected.

Validation of anti-TEM1 and 5 antibodies for imaging of tumor neovasculature in a GBM mouse model

Collin M. Torok, MD, Mrudula Pullambhatla, MS, , Martin G. Pomper, MD, PhD, , Catherine A. Foss, PhD
Johns Hopkins Hospital

PURPOSE: Tumor endothelial markers (TEMs) are proteins reported to be selectively expressed in tumor neovasculature. The purpose of this study was to evaluate anti-TEM1 and anti-TEM5 antibodies in an intracranial U87 glioblastoma multiforme mouse xenograft model as potential probes for in vivo visualization of active tumor border.

METHODS: Anti-TEM1 and anti-TEM5 antibodies were evaluated in cultured cells, frozen U87 xenograft sections, and imaged in vivo with SPECT-CT and near-infrared fluorescence (NIRF) imaging. Anti-TEM antibodies were labeled with fluorescent dyes and tested along with anti-CD31 antibody (vascular endothelial marker) in U87 xenograft sections. SPECT-CT and NIRF imaging were performed with radiolabelled (I-125) and fluorescent dye labeled anti-TEM antibodies. Ex vivo tissue autoradiography and immunofluoresence microscopy (IMF) were subsequently performed. A biodistribution study included mice injected with [125I] anti-TEM1 antibody (n=10), [125I] anti-TEM5 antibody (n=10), and isotype control (n=8) with 48 and 72-hour data collection time points. NIRF imaging and IMF were performed on subcutaneous PC-3 prostate cancer and H1650 lung cancer mouse xenografts.

RESULTS: Dual-labeled (I-125, IRDye) anti-TEM5 antibody successfully grossly delineated an intracranial U87 glioma using in vivo optical imaging and ex vivo autoradiography. Anti-TEM5 antibody also successfully defined subcutaneous prostate cancer xenografts and their lymph node metastases at optical imaging. On IMF, anti-TEM5 antibody exhibited a diffuse cytoplasmic staining pattern that was nonspecific for neovasculature. Dual-labeled anti-TEM1 antibody successfully delineated an intracranial U87 glioma using in vivo optical imaging, with a specific co-localization pattern with tumor neovasculature on IMF. Isotype control antibody showed mild focal uptake in a U87 xenograft, which was likely nonspecific and secondary to local tumor-associated inflammation given no significant accumulation on ex vivo IMF. SPECT-CT and biodistribution data did not show consistent uptake in the inoculated hemisphere, most likely due to the heterogeneous distribution of tumor size and associated tumor-specific vasculature. NIRF imaging of the prostate cancer model mice revealed anti-TEM1 antibody uptake in a pattern consistent with neovasculature in the viable tumor rim, whereas anti-TEM5 antibody strongly bound to all regions of the tumors.

CONCLUSION: These data show that both anti-TEM1 and TEM5 antibodies sensitively and specifically detect GBM and prostate cancer xenografts using in vivo optical imaging and that anti-TEM1 antibody more specifically localizes to tumor neovasculature. However, SPECT-CT and biodistribution data did not demonstrate corresponding significant findings. NOTE: This represents a study in progress not yet presented elsewhere and will be submitted as a formal manuscript in July (grantee first author).

Image-Guided Therapies & Interventions

Nanodiamond-Gadolinium (ND-Gd) Coupling to Catheter Surfaces for Enhanced Device Visualization

Erik Robinson, Dean Ho, Tom Meade, Lisa Manus, Daniel Mastarone
Northwestern University

Utilization of nanomaterials stands to impart tremendous gains in the medical diagnostic and imaging fields. Carbon based nanoparticles, in particular Nanodiamonds (NDs), have been singled out due to their biocompatibility and relative ease with which their surface can be functionalized. Previous work has revealed their ability to improve the relative relaxivity of certain contrast agents. In particular, Nanodiamond-Gadolinium (ND-Gd) conjugates have shown promise in enhancing interventional MRI imaging potential. Thus, carbon based NDs provide a multifunctional platform within the ever increasing field of Nanomedicine. Resultantly, an effort to physically link ND-Gd conjugates to the surface of a polymer coated catheter was attempted. The polymer surface was chemically modified to facilitate the reaction of ND-Gd conjugates. Vacuum plasma oxidation resulted in an increase in surface oxygen content. Subsequently, silinization of the oxygen rich surface by means of an amine (-NH) terminated silane produced an amine rich substrate. Finally, ND-Gd conjugates, presenting predominately carboxyl (-COOH) groups, were to be linked to the catheter surface. Each successive reaction step was validated utilizing X-ray Photoelectron Spectroscopy (XPS). Elemental analysis of modified catheter surfaces revealed the apparent changes in chemical signatures indicative of the respective chemical reactions. Continued work will improve the reaction efficiency and concentration of ND-Gd conjugates onto modified catheter surfaces. Further modification of medical related devices or instruments will provide clinicians a greater degree of flexibility during interventional procedures and ultimately improve patient outcomes.

All-Fiber Optic Endoscopic Catheter System for Simultaneous OCT and Fluorescence Imaging

Jessica Mavadia, Jiefeng Xi, Yongping Chen, Xingde Li
Johns Hopkins University

We present an all-fiber-optically based endoscopic catheter for simultaneous fluorescence imaging and optical coherence tomography. This design entails the use of double clad fiber in the endoscopic catheter for delivery of excitation light for both optical coherence tomography (OCT) and fluorescence imaging while collecting the OCT signal through the single-mode core and fluorescence emission through the large inner cladding. Additionally, a custom 2x1 combiner was utilized to integrate both systems for an entirely fiber optic based system. We have demonstrated concurrent imaging intraluminally in ex vivo rabbit esophagus where we were able to visualize fluorescence distribution along with morphological information gathered from OCT suggesting its utility in clinical applications.

A semi-automated vascular access system for preclinical models

Brittany N. Berry-Pusey, Yen-Chi Chang, Stephen W. Prince, Kevin Chu, Waldemar Ladno, John David, Robert Silverman, Richard Taschereau, David Stout, Tsu-Chin Tsao, Arion Hadjioannou

University of California, Los Angeles

Purpose: Preclinical molecular imaging technologies have an increasingly broader application base while at the same time are becoming more user-friendly. Mouse tail vein injections are a routine but critical step in most imaging applications, with poor injections greatly affecting the experimental results. The high and specialized skill set required to perform good tail vein injections leave many preclinical imaging scientist ill-suited to perform this task. In fact, in a recent study we found routine injections left 1-50% of the injected probe in the tail tissue. The high amounts of the injected probe remaining in the tail tissue, along with the large variations, was alarming. To reduce the skill set required for tail vein injections and to improve the accuracy and consistency of the tail vein injections, we have developed a semi-automated vascular access system (VAS).

Methods: To use the system, a user secures the tail of an anesthetized mouse to the heated VAS tail holder. NIR light, cross polarizers, and a CCD camera are used to image the tail. The image is processed with a bandpass filter and edge detection methods are used to detect the location of the tail vein. Once the vein location is identified, a needle is moved to the injection site by computer-controlled motors. The needle penetrates the tail tissue and enters the vein. A pressure transducer attached to the needle indicates when the needle has reached the vein and signals to the motor to stop further motion. With the needle in the vein, the desired probe can be administered via a syringe pump.

Results: Individual components of the VAS; including the imaging and the pressure transducer feedback to the motor, were tested with a mouse tail phantom. After the individual components were validated, the integrated VAS was assembled and used for the injection of radioactive probes for the positron emission tomography (PET) imaging of mice. These studies showed that the accuracy of the device, as measured by the percentage of injected probe left in the tail, is approximately 17% (+/- 6.8%).

Conclusion: To decrease the skill set required to perform mouse tail vein injections and to increase the accuracy and consistency of injections, we have designed, built, and tested a preclinical vascular access system. We continue to optimize the VAS prototype.

Multi-Modality Rigid and Non-Rigid Prostate Image Registration to Whole- Mount Histology

Samantha L. Lipman, Christina M.L. Hsu, Mark L. Palmeri, Thomas J. Polascik, Matthew S. Davenport, Christopher Kauffman, Rajan T. Gupta, Evan Kulbacki, John Madden, Kathryn R. Nightingale

Duke University

PURPOSE: Prostate Cancer (PCa) is the most common non-skin cancer among American men. B-mode ultrasound (US) is currently used to guide needle biopsies, but US images suffer from poor prostate structure visualization. Acoustic Radiation Force Impulse (ARFI) imaging is being developed to guide needle biopsies and focal therapies in the prostate. Magnetic Resonance (MR) imaging is also an emerging imaging modality that can delineate structures and characterize regions of disease. We are developing image registration techniques that facilitate correlation of in vivo ARFI, B-mode US, and MR images obtained prior to radical prostatectomy with whole mount histology data after ressection.

METHODS: US and ARFI imaging were performed pre-operatively using a side-fire ER7B endorectal ultrasound probe on a Siemens ACUSON SC2000 ultrasound scanner and a custom transducer rotation stage. Pre-operative standard T1 and T2 MR images were also obtained, along with Diffusion Weighted Imaging (DWI) and Apparant Diffusion Coefficient (ADC) MR imaging sequences. 3D models of the prostate were formed by segmenting pathology and internal prostate structures using itk-SNAP and Altair HyperMesh. Non-rigid registration of the different models was performed using Advanced Normalization Tools (ANTs), and the registered images were evaluated for co-localization of confirmed pathology.

RESULTS: This multi-modality image registration methodology was validated using simulated prostate anatomy and finite-element techniques and found to improve the accuracy of the average displacement of registration markers by 76% in the MR simulation and 58% in the US simulation. In vivo ARFI images delineate internal structures of the prostate with higher contrast than the B-mode US images. The multi-modality image registration has demonstrated correlation between regions of decreased displacement in ARFI images with prostate cancer as diagnosed with whole-mount histology and MR imaging. Atrophy of prostate tissue has also been associated with regions of decreased displacement in ARFI images.

CONCLUSION: Confirmed PCa pathology was found to align with similarly suspicious regions in both ARFI and MR images. With its improved anatomical visualization over traditional B-mode imaging, ARFI imaging holds promise for providing targeted image guidance of prostate focal therapy and needle biopsy.

Accurate High-Intensity Focused Ultrasound Ablation in a Porcine Liver Model through Integration of Real-Time Image Guidance, Robotic Navigation, and Elastographic Monitoring

Daniel A. Carnegie, Emad M. Boctor, Xiaoyu Guo, Hyun-Jae Kang, Nishikant Deshmukh, Pezhman Foroughi, Everette Burdette, Chris Diederich, Robert J. Webster III, Jessica Burgner, Michael A. Choti

Johns Hopkins School of Medicine, Div. of Surgical Oncology

Purpose: Active research into accurate, minimally-invasive, image-guided destruction of focal hepatic malignancies will expand the population of treatable patients and potentially extend survival. Our objective is to develop an accurate, real-time monitored, minimally-invasive ablation system through the integration of high-intensity focused ultrasound (HIFU) ablation within a flexible needle configuration, robotic steerable active cannula guidance, and real-time elasticity monitoring.

Methods: HIFU is an ablative therapy that utilizes therapeutic ultrasound to thermally destroy tumors. Peri-procedural image guidance is critical to effective use of this modality to both plan and monitor effective ablation. Though conventional ultrasound and MRI are most frequently used for image guidance, they are imperfect for visualization targeting. Precise control of needle placement through robotic guidance can improve targeting of therapy. In addition, improved real-time ablation monitoring using real-time elasticity imaging offers the opportunity to modify and confirm accurate delivery of therapy. In this study, we have adapted ultrasound strain imaging to provide accurate real-time image guidance for HIFU needle ablation

Results: Successful engineering of integrated precise robotically-controlled steerable (active cannula) needle ablator with real-time elasticity image-guidance allowed ablation of hepatic lesions in ex vivo liver models. We also showed a successful utility of guiding the ablation tool using robotic steerable active cannula in an in vivo porcine model. Strain imaging allowed for the assessment of lesion ablation and will facilitate assessment of completion of treatment. The engineered robot-controlled acoustic ablator provided precise targeting control when coupled to real-time image guidance.

Conclusions: These experiments demonstrated that targeted liver ablation with HIFU is feasible and safe with homogeneous and shaped ablation sites. Robotic control of the active cannula delivery of the needle ablator allows for accurate, precise targeting in an in vivo environment. The ablation can be coupled with elastography which provides complementary tissue strain characteristics to determine completeness of treatment. We intend continue to fully integrate real-time imaging modalities with robot-controlled active cannula acoustic needle ablator for complete image-guided minimally invasive treatment of liver tumors.

Image Processing, Displays & Perception

Localizing dysplasia for digitally-aided prognosis of Barrett's Esophagus

Virginia M. Burger
University of Pittsburgh

Purpose: Barrett's Esophagus leads to esophageal adenocarcinoma in 0.1% of patients per year. However, as dysplasia occurs and increases in Barrett's Esophagus tissue, cancer risk increases from 0.5% to 10% for patients with high-grade dysplasia. The sooner dysplasia is diagnosed, the sooner treatment can begin with the intention of preventing progression from dysplasia to cancer. We analyze biopsies of Barrett's esophagus patients with diverse levels of dysplasia over a five-year period. With the greater goal of developing a computational prognosis model for cancer risk, here we focus on intermediate representation of scanned esophagus tissue biopsies. Our images are labeled with a diagnosis from a pathologist, ranging from no dysplasia to cancer. In future work, we will determine features from the images to use in algorithms for cancer prognosis. However, as it often occurs that only a small portion of tissue was used to make the diagnosis, and that a single biopsy may contain glands with both zero and high-grade dysplasia, we must first segment the tissue into regions with similar degree of dysplasia.

Methods: The scanned biopsies are labeled with cancer biomarkers, which we measure on individual nuclei. A typical biopsy in our data set is 6000x6000 pixels, necessitating algorithms for large images. We create a network in which each nucleus in the image is a node, and edges between nodes are weighted according to biomarker similarity. By performing a random walk on the network, clusters of nuclei with similar intensity patterns emerge. As the biomarkers are related to dysplasia on the nuclei, these clusters will also have similar degrees of dysplasia.

Results: The random-walk based clustering identifies regions with similar levels of dysplasia. In addition, portions of healthy esophagus and stomach inappropriately contained within the Barrett's esophagus tissue biopsy are detected.

Conclusion: By organizing regions of tissue on each slide according to their biomarker similarity, we are able to find regions of tissue with similar degrees of dysplasia. This intermediate step provides insight into cancer development and perception: is dysplasia uniform across the sample, or is it localized to a small region? How much dysplasia must be present, or how wide-spread must it be, for a pathologists to make a given diagnosis? Future work will use these homogeneous tissue regions to determine features for cancer prognosis in Barrett's esophagus patients, as we approach our goal of developing an algorithm to identify regions of interest for cancer prognosis.

Improved T1 Mapping Accuracy by Accounting for the Flip Angle Profile in SSFP Imaging

Mitchell Cooper, Thanh Nguyen, Pascal Spincemaille, Martin Prince, Jonathan Weinsaft, Yi Wang

Cornell University; Weill Cornell Med. College

Purpose: In MRI T1 relaxation time can be described as the amount of time it takes for the longitudinal magnetization of the proton spins to recover to their equilibrium state. The T1 times of tissues can be measured in-vivo and used as a metric to identify diseased tissues. Previously, the Steady State Free Precession (SSFP) pulse sequence has been used to measure T1 in cardiac applications to assess the myocardium. T1 time can vary with infiltrative cardiomyopathies such as amyloid. However, previous SSFP techniques fit for T1 assuming a constant RF excitation profile, which is not a valid assumption for fast two dimensional imaging. Here we developed a technique to correct fits for SSFP T1 quantification by accounting for the imperfect RF excitation profile through Bloch simulations. We tested our method in phantoms and in skeletal muscle for validation.

Methods: An inversion recovery 2D Look-Locker SSFP pulse sequence with continuous readout was implemented on a GE 1.5T scanner to measure T1. A Hamming-windowed half-sinc RF profile was used for all scans. Magnesium Chloride phantoms were imaged with the SSFP sequence at flip angles of 30, 60 and 90 degrees. In addition, spin-echo scans were done to obtain reference standards for T1 and T2. Healthy volunteers (n=6) were scanned in accordance with IRB regulations. Imaging was done with a 60? flip angle in the thigh to allow for the long spin-echo inversion recovery scan to be carried out in-vivo.

Results: T1 and T2 values of the Magnesium Chloride phantoms from the reference standard spin echo methods were 304/30, 589/67, 919/121, 1532/275 ms. Fitting the Look-Locker SSFP data with our correction technique in phantoms provided errors less than 5.8% for flip angles of 30?, 60? and 90?, while T1 error could be as high as 22.7% when fitting without RF profile correction. In-vivo RF profile correction at 60? with a TR = 18 ms provided excellent T1 measurements (-0.6 ± 1.8% error) indicating a 94% improvement in accuracy compared with results obtained without profile correction.

Conclusions: Using our fitting algorithm to account for the imperfect RF profile, T1 values in phantoms and in-vivo were more accurate compared to fitting with a constant RF profile. By using Bloch equations, our fitting method can easily account for the RF profile as well as different magnetization preparations. Future studies can look at the inclusion of B0 and magnetization transfer correction in our T1 fits.

Atlas-Based MR Inhomogeneity Correction

Kwame S. Kutten, Johnny T. Hsu, Susumu Mori, Michael I. Miller
Johns Hopkins University

Purpose: MR images of the brain are often corrupted by a smooth spatial distortion of intensity referred to as an inhomogeneity field or a bias field. These artifacts can cause automatic image analysis algorithms to fail and therefore must be removed.

Methods: The Parametric Bias Field Correction (PABIC) method was introduced to remove inhomogeneity artifacts from MR images. In PABIC, how well an estimated bias field fits an image is determined by the image energy function. Every pixel "sees" the same energy function regardless of its location [2]. While this framework is sufficient in most cases, spatial prior information can greatly improve its performance. Therefore the energy function was redefined and tissue-specific probabilistic atlases were created from the brains of 14 healthy subjects. These atlases were then used to weigh the energy function depending on a pixel's location. Atlas-based bias correction was performed with an automated cyclic pipeline. First, affine registration followed by diffeomorphic registration (LDDMM) [1] were used to align the subject to the atlases. Next, bias field estimation was done in atlas-space using the probabilistic atlases. Finally, the original image was corrected after applying the inverse registration transform to the estimated bias field. This bias-corrected image was then used as the pipeline's new input completing the cycle. Three rounds of registration/bias-correction were applied to refine the results.

Results: Qualitative and quantitative comparison of the images before and after bias correction showed that our method reduced the effects of MR inhomogeneity. In image histograms, the peaks became narrower and the standard deviations in tissue-type intensities decreased. This indicated that tissue intensities became more consistent throughout the images.

Conclusions: The PABIC algorithm was modified to use tissue-specific probabilistic atlases. A cyclic pipeline was developed to map the subject to atlas-space for bias field estimation and the bias field back to subject-space for bias field correction. The current atlases were designed for skull-striped images. Further work is needed to generalize this method to images that have not been skull-striped.

References [1] M. F. Beg, M. I. Miller, A. Trouve, and L. Younes. Computing large deformation metric mappings via geodesic flows of diffeomorphisms. International Journal of Computer Vision, 61(2):139-157, 2005. [2] M. Styner, C. Brechbuhler, G. Szekely, and G. Greig. Parametric estimate of intensity inhomogeneities applied to MRI. IEEE Transactions on Medical Imaging, 19(3):153-165, March 2000.

Breathing a little easier, or not: tracking and analyzing lung changes with CT & PRM

Jennifer L. Boes, Meilan K. Han, Charles R. Meyer, Alnawaz Rehemtulla, Ella A. Kazerooni, Fernando J. Martinez, Brian D. Ross, Craig J. Galbán
The University of Michigan

Purpose: Chronic obstructive pulmonary disease (COPD) is an increasingly recognized public health problem, with rising morbidity and mortality. Current tools for analysis and tracking of lung state and function are simple and straightforward, but often cannot differentiate varying pathobiology in this highly heterogeneous disorder. Image-based biomarkers sensitive to COPD sub-types would open the possibility for individualized treatment, thus improving patient outcome.

Methods: We analyze parametric response mapping (PRM) on whole lung CT inspiration-expiration pairs to determine its sensitivity as a biomarker for lung function. PRM uses voxel-wise analysis of image pairs to measure localized change. Inspiration and expiration scans are co-registered using a deformable mapping prior to PRM analysis. Single time-point data consisted of paired inspiration and expiration CT scans for 194 COPD patients from the COPDGene Study. We also applied PRM to multi-time-point lung CT data for a limited cohort of 18 patients.

Results: In the COPDGene data, PRM metrics for emphysema and small airways disease (fSAD) were found to be significant parameters for modeling FEV1 (a standard pulmonary function measure). Longitudinal CT lung PRM is consistent with early results, while also highlighting the need for consistently acquired imaging.

Conclusions: PRM provides a versatile imaging marker capable of diagnosing disease extent and phenotype, while providing spatial information of disease distribution and location. PRM's ability to distinguish between lung phenotypes will aid in more accurate diagnosis of patients than current pulmonary function tests (PFTs) and CT-based metrics.

Myocardial Lesion Detectability in PET Scan

Eugene S. Mananga, Jinsong Ouyang, Ali A. Bonab, Yu-Hua D. Fang, Georges El Fakhri
Massachusetts General Hospital; Harvard Medical School

PURPOSE: The aim of this work is to characterize myocardial lesion detectability when performing a myocardial positron emission tomography (PET) scan and assess changes in lesion detection as a function of variable physiological uptake parameters such as different Background-to-Myocardium, Liver-to-Myocardium, Lung-to-Myocardium, and Lesion (Defect in perfusion)-to-Myocardium concentration ratios. These varying ratios are used to assess performance in myocardial ischemic lesion detection for different scenarios relevant to the clinical setting. We mimic ischemic (cold) lesion-present studies by combining lesion-only and lesion absent (normal) studies and use a mathematical observer, the channelized Hotelling observer (CHO), as a surrogate of human observer, for the task of lesion detection in a signal-known-exactly/Background-known-exactly paradigm.

METHODS: All phantom experiments were performed on a PET-CT scanner at Massachusetts General Hospital. Each compartment (myocardium, myocardial lesion, liver, lung, background) of the phantom was filled individually and sequentially with 18F and scanned separately using clinical acquisition protocols (sampling, tomographic reconstruction, etc.) in order to extend our findings to clinical studies. This yielded studies of a single organ or compartment that were next combined to generate multiple studies with different uptake ratios as seen usually in clinical cardiac PET studies. These ratios were determined based on actual cardiac PET studies performed using 18F flow agents currently in Phase-III trials. 16 Noise realizations (Poisson noise) were generated for each set of projections of a composite study. Data combination and image reconstruction were performed using Siemens e7 tools, as well as algorithms developed in our lab to allow incorporation of lesions within our scans with the correct attenuation and normalization factors, as well as the scanner variable point spread function. Gaussian filter, one energy window (435-650 keV) and point spread function – ordinary poisson – ordered subsets expectation maximization (PSF-OP-OSEM) were used for reconstruction with 2 iterations and 8 subsets.

RESULTS: Our results demonstrate that the lesion detectability is not affected by change in concentration ratio inputs of Background/Myocardium, Liver/Myocardium, and Lung/Myocardium. However, change in the concentration ratio input of the defect/Myocardium lead to a significant change of the contrast. Results also show that about 20 million counts give optimize signal with low noise.

CONCLUSION: Cold lesion detection is a more complex task than hot lesion detection and is still not completely characterized. We have designed an approach that involves generating a realistic phantom based on patient data to generate lesion-present studies that allow us to characterize the detectability of cold lesions with a numerical observer, the CHO.

Distinction Between Normal White Matter And Glioma Infiltration By SS-OCT In Human Ex Vivo Tissue

Carmen Kut, Shaan Raza, Jiefeng Xi, Jessica Mavadia, Hugo Guerero-Cazares, Elliot McVeigh, Xingde Li
Johns Hopkins University

Abstract: INTRODUCTION: There is a need to better delineate and resect glioma margins, especially at the transitional, infiltrative zones between tumor and white matter. Optical coherence tomography may provide a non-invasive, high speed and micrometer resolution imaging technique that may prove effective in intraoperative settings.

METHODS: We studied optical attenuation characteristics and OCT features in intra-operatively obtained ex vivo tissues from 12 glioma (grade I-IV) and 5 control patients (temporal lobectomy). Analysis was performed on all samples using OCT with ~16.0 and 9.0 μm resolution (lateral x axial) and 1-3 mm imaging depth. Intensity values from each homogeneous tissue section were processed and exponentially fitted to yield the optical attenuation coefficients.

RESULTS: We observe a statistical significance for the average attenuation coefficients between glioma infiltrated zones and normal white matter tissue ($p = 0.03$ to 0.05). Sensitivity/Specificity analysis of normal white matter versus infiltrated zones (grade II-IV) reveals a cut off values of 4.5/mm with 94% sensitivity and 99% specificity. In addition, we identified two tumor features that can be clearly identified under the SS-OCT system: 1) Microcysts in 75% (3/4) of our grade I-III glioma samples and 2) necrosis and palisading features in 100% (8/8) in our grade IV glioma samples.

CONCLUSION: To our knowledge, there has been neither comprehensive nor application of the performance and utility of optical imaging techniques in guiding glioma resections. This study provides a baseline study for future studies in establishing sensitivity and specificity of optimal imaging techniques in identifying and resecting glioma infiltrative regions in the OR.

An Image Derived Input Function for Simultaneous Neurological MR/PET Imaging

Ciprian Catana
Harvard Medical School

Purpose: In order to accurately estimate biologically relevant information (e.g. kinetic parameters, binding potentials, cerebral metabolic rate of glucose [CMRglc]) from dynamic PET data, the plasma time activity curve, or input function (IF), is necessary. The gold standard for measuring the IF is through arterial sampling (AIF) performed throughout the PET scan; however, this method is invasive in nature. Here we present a non-invasive method which used both the PET and the simultaneously acquired MR data.

Methods: Healthy volunteers underwent simultaneous MR-PET imaging with the BrainPET, an MR-compatible brain PET scanner able to operate inside a Siemens 3T TIM Trio MR scanner. Subjects were injected with ~5 mCi F18-FDG and scanned for 90 minutes. The radial artery was catheterized to allow for simultaneous blood sampling over the imaging period and samples were collected at every 5 seconds for the first 2 minutes then at 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes post injection which was used as the gold standard for comparison of the IDIF. The vasculature was derived using the customarily acquired high resolution anatomic MR image (MR-MPRAGE) and a small field-of-view Time-of-Flight (TOF) MR-angiography sequence, allowing for the segmentation of both small and large arteries of the head. Due to intensity inhomogeneities in the TOF data and slab boundary artifacts, simple thresholding does not yield accurate arterial segmentation; rather, the TOF images first underwent morphologic top-hat filtering. A high threshold was applied to a rectangular region in the center of the TOF volume to capture some arterial segments. These segments were then used as a seed for a region-growing algorithm. To extend the mask into the carotid region, the TOF derived mask was used as a seed for another region growing algorithm with the MPRAGE as the image volume.

Results: Vascular structures were accurately segmented with out dual-sequence method and the arterial mask was found to be robust to subject motion. Changes in CMRglc was found to be on the order of 1.55%±0.23% when using our IDIF method. Total subject scan time was increased by 6.27 minutes.

Spatial heterogeneity of patterns of cortical amyloid deposition in aging and its relationship to me

Rachel Aine Yotter, Jimit Doshi, Vanessa Clark, Jitka Sojkova, Yun Zhou, Dean F. Wong, Luigi Ferrucci, Susan M. Resnick, Christos Davatzikos
University of Pennsylvannia, Section of Biomedical Image Analysis

Background: The recent development of amyloid imaging compounds has allowed in vivo PET imaging of amyloid. Generally, sensorimotor and occipital areas remain relatively free of amyloid, while the frontal lobe and precuneus are affected early (Braak 1997). Using a new methodology for approximating spatial patterns of temporal progression of plaque deposition from cross-sectional images, we demonstrate similar patterns using 11C-PiB (Pittsburgh Compound B) PET data from the Baltimore Longitudinal Study of Aging (BLSA). Moreover, spatial patterns were significantly different between memory decliners and non-decliners.

Methods: To extract the spatial dynamics of amyloid deposition, participants were ranked based on their individual cortical distribution volume ratio (cDVR) score, resulting in an across-group waveform for each voxel such that the x-axis is the participants' cDVR score and the y-axis is the DVR value associated with that voxel across the group. When these waveforms were examined, the general pattern was the relative absence of DVR increase, followed by a linear increase in voxel-wise DVR with respect to cDVR value (Sojkova 2011). 64 subjects (35 men, age 76.61±6.89 years) from the BLSA neuroimaging sub-study were included. Each voxel waveform was fitted using a piece-wise linear fit. Due to spline smoothing of the data inherent in the fitting process, individual deviations from the assumption of less amyloid deposition earlier in disease progression have less effect over the general pattern. Furthermore, we analyzed subjects grouped into top and bottom 20% California Verbal Learning Test (CVLT) slopes. The significance was quantified using a permutation test on sensorimotor and temporal ROIs.

Results: Spatial patterns demonstrate relative sparing of sensorimotor and occipital areas, while the frontal lobe and precuneus begin to accumulate amyloid earlier, i.e., need minimal cDVR before they begin to be affected. The spatial patterns for CVLT subgroups diverge, such that the "cognitively stable" subgroup had relative sparing of sensorimotor and temporal areas compared to the "cognitively declining" group. Permutation testing revealed that left hemisphere differences between subgroups are significant ($p < 0.05$, corrected for multiple comparisons using FDR).

Conclusions: We have quantified spatial patterns of progression of amyloid deposition in older adults. When our approach was applied to subgroups based on cognitive performance, the spatial patterns diverged. This finding has implications for prediction of cognitive decline from amyloid data, suggesting that it is the spatial pattern rather than total amyloid burden that may be more relevant.

Searching in three dimensions: How do radiologists move their eyes when viewing Chest CTs?

Trafton Drew, Melissa Le-Hoa Vo, Francine L. Jacobson, Steven E. Seltzer, Jeremy M. Wolfe
Brigham and Women's Hospital

Purpose: We understand a great deal about how visual search is carried out in 2D scenes and medical images (e.g. Kundel et al., 2007). Very little is known about how search is accomplished in stacks of 2D images representing a 3D volume. How do radiologists search such stimuli? More importantly, how does that search behavior relate to errors?

Methods: 10 trained radiologists searched lung CT scans in stack mode while we monitored their eye-movements. Each radiologist had 3 minutes to read each of 8 lung CT cases. The cases were taken from the Lung Image Data Consortium, which provided the precise location and volume of all chest nodules. Radiologists marked nodules with a mouse click. Eye-position in X/Y space was recorded at 1000Hz and co-registered with slice/depth plane as the radiologist scrolled through the lung, allowing a representation of eye position in the 3D volume of the lung.

Results: Radiologists varied widely in the pattern of movements through slices with some making a single pass while others repeatedly moved up and down through the stack. Regardless, all tended to hold their eyes fixed in the XY plane whenever they moved in depth. In typical studies of errors in 2D images, it is reported that there is a roughly equal proportion of missed lesions that are never fixated (search errors), fixated briefly (recognition errors), or fixated extensively (decision errors) (c.f. Krupinski & Nishikawa, 1997). In the 3D case, we found an elevated proportion of search errors - 47% of missed nodules were never fixated, 39% were briefly fixated, while only 14% were long-fixation, decision errors.

Conclusions: Understanding the source of errors is critical to improving radiologist performance. While different radiologists appear to adopt different search strategies and these strategies are consistent across multiple cases, it is not yet clear whether one search strategy is superior. The relatively high rate of nodules that were never fixated may reflect a form of a “satisfaction of search” error specific to stacks of 2D images. It is possible that radiologists overestimate the degree to which they have searched a given case or underestimate the level of close scrutiny necessary to detect lung nodules. In either case, feedback about eye position may serve as a useful training device for future radiologists.

Imaging Features Associated with Malignant Foci on Breast MRI

Myers KS, Kamel I
Johns Hopkins Hospital

Purpose: To identify imaging and clinical characteristics associated with malignant foci on breast MRI.

Methods: Institutional review board approval was obtained for this retrospective, HIPAA-compliant study. A departmental breast MRI database was queried to identify foci. 137 foci in 109 patients having biopsy results or at least two years of clinical follow-up were eligible and imaging and clinical characteristics were evaluated. Imaging characteristics assessed included: foci size, enhancement kinetics, morphology, visibility on the maximum intensity projection (MIP) image, conspicuity, and number of foci as well as abnormal ipsilateral axillary lymph nodes, background uptake of contrast and breast density. Clinical characteristics included: age, reason for the exam and risk factors for breast cancer. Benign or malignant outcome was determined by pathology or at least two years of clinical follow-up.

Results: 9/137 (6.5%) of all foci, those that were followed and those that were biopsied, were malignant. All foci with malignant pathology were selected for biopsy, with the malignancy rate of 9/47 (19.1%) in the biopsy group compared to a rate of 0/90 (0%) in the group that was followed. Characteristics that were associated with malignant foci were: washout kinetics, larger size, very dominant foci, minimal background uptake and abnormal ipsilateral axillary lymph nodes. No foci less than 3.5mm in size were malignant.

Conclusion: Several imaging features were associated with malignant foci and can guide in the selection for biopsy.

Bioinformatics

A whole genome assembly of the Black-breasted Hillstar (Oreotrochilus melanogaster)

Jessica Weber, Jeremy Edwards, Christopher Witt
University of New Mexico

PURPOSE: This study compares several open-source programs in the assembly of the Black-breasted Hillstar (Oreotrochilus melanogaster) hummingbird genome. Traditional assembly methods are too computationally intensive to be feasible for the assembly of most animal genomes. However, a variety of de Bruijn graph assembly programs are now available that allow genomic assemblies to be completed in a short amount of time on relatively modest hardware. For species with a closely related reference sequence, typically about 80 percent sequence identity, reference-guided assemblies have been shown to increase the quality of the assembly. For those species without an available reference sequence, de novo sequence assembly is used. The nearest reference genome to the Black-breasted Hillstar has roughly 68 percent sequence identity. Since most species share less than 80 percent sequence identity with a reference, this study provides a more realistic comparison of the two assembly methods. While the number of completed genomes continues to increase, only two avian genomes are currently available: the chicken (Gallus gallus) and the zebra finch (Taeniopygia guttata). Hummingbirds represent the second largest avian family and have been reported to have the smallest avian genomes. Their intense oxygen requirements also make them a primary model for the study of high-elevation adaptation. As the first hummingbird genome sequenced, the Black-breasted Hillstar genome will be compared to the available chicken and zebra finch genomes to identify major genomic differences between these taxa. Additionally, the high altitude Black-breasted Hillstar genome will be used to align the 30X coverage sequences of three lowland species (the Hairy Hermit hummingbird (Glaucis hirstus), the White-necked Jacobin hummingbird (Florisuga mellivora), and the Vaux's Swift (Chaetura vauxi)). These sequences will then be used to examine the molecular basis of high-elevation adaptation in the Black-breasted Hillstar.

METHODS: 100X sequencing was completed using paired end reads on an Illumina HiSeq next-generation sequencer. Reference guided assembly was completed using the available zebra finch genome using the Burrows-Wheeler Aligner (BWA) and Bowtie aligner followed by the Velvet assembler using the reference-guided assembly option. De novo sequence assembly was completed using the Velvet and ABySS assemblers. The Gap5 assembly viewer and editor and Integrative Genomics Viewer (IGV) were used to view and manually evaluate the assemblies. Amosvalidate, part of the AMOS package, was used as an additional validation of the sequence assemblies.

RESULTS & CONCLUSIONS: A comparison of reference guided and de novo assembly using several open-source programs will be presented.

Aortic Wall Thickness: An Independent Risk Factor for Aortic Dissection?

Hadas Shiran, MD, Justin Odegaard, MD, PhD, Gerald Berry, MD, D. Craig Miller, MD, Michael Fischbein, MD, PhD, David Liang, MD, PhD
Stanford University

Aortic Wall Thickness: An Independent Risk Factor for Aortic Dissection in Connective Tissue Disorders?

Purpose: While aortic aneurysm size is known to portend a higher likelihood of aortic complications in patients with connective tissue disorders (CTD), other objective tools are needed to determine which patients are at greater risk of dissection, especially those which reflect structural integrity and strength of the aortic wall. We evaluated aortic wall pathology in CTD patients with and without acute aortic dissection to search for parameters that affect risk of dissection.

Methods: Retrospective review of ascending aortic pathology from patients with Marfan Syndrome (MFS, n=53), bicuspid aortic valve (BAV, n=20), and Loeys-Dietz Syndrome (LDS, n=8) without dissection undergoing prophylactic aortic root surgery were compared to patients with acute type A aortic dissection (AAAoD, n=16). Controls were cadavers without cardiovascular cause of death (n=13). Aortic wall medial wall thickness was measured. Presence and severity of medial myxoid degeneration (MMD) and elastin loss and fragmentation were graded using published scales.

Results: Of the MFS patients undergoing prophylactic repair the average aortic medial wall thickness at the level of the Sinuses of Valsalva was 813 ± 216 microns, whereas it was 782 ± 190 microns for the BAV patients and 623 ± 132 microns for the LDS patients. The mean aortic root diameter did not correlate with medial aortic wall thickness in patients without AAAoD. In patients with acute dissection, the mean aortic medial thickness was 487 ± 458 microns. For the controls, mean aortic medial thickness was 1745 ± 360 microns. All CTD subgroups had thinner medial walls than controls ($p < 0.001$). Comparison amongst subgroups showed a thinner medial wall in AAAoD than MFS ($p = 0.02$) and BAV ($p < 0.001$) subgroups, but the medial wall was very thin in LDS patients and not significantly different from AAAoD patients. Degree of MMD was more severe in MFS, LDS, and AAAoD than in BAV patients. Elastin loss did not vary significantly between CTD groups.

Conclusions: Thinner aortic wall medial thickness may be linked to aortic dissection. High resolution imaging techniques in the future may make morphological assessment of aortic medial wall thickness in vivo a reality, which theoretically could provide more refined risk prognostication for acute aortic dissection.

Modeling / Simulations

Hemodynamic characterization of Aortic Valve Bypass Surgery using CFD models based on MRA and PCMR

Adrian Lam, BS, Muralidhar Padala, PhD, Vinod Thourani, MD, John Oshinski, PhD
Georgia Institute of Technology

Aortic Valve Bypass Surgery (AVBS) is an option for patients who suffer from both aortic valve stenosis and severe ascending aortic calcification. In AVBS, a conduit containing a prosthetic valve is introduced into the LV transapically and is attached to the descending thoracic aorta. However, some AVBS patients suffer from cerebral events, and intra-aortic thrombus has been reported. The objective of this study is to use Magnetic Resonance Angiography (MRA), Phase Contrast Magnetic Resonance (PCMR), and Computational Fluid Dynamics (CFD) to understand the hemodynamics of AVBS in order to correlate how flow patterns may relate to post-surgical complications. 20 patients received a follow-up MRI scan 6-12 months after surgery. Contrast-enhanced MRA was used for patient-specific 3D geometry reconstruction. PCMR images were acquired at the conduit, across the subclavian/carotid arteries, and at multiple locations along the descending thoracic aorta. Average velocities were obtained from segmented vessels at the native aortic valve and the conduit on PCMR images for inlet boundary conditions. Patients with retrograde flow in the descending thoracic aorta were selected for modeling. All CFD models were simulated with Fluent ANSYS, Inc. and post-processed using Tecplot. Streaklines and streamtraces based on the CFD generated flow field show that the arch vessels are supplied with blood from both the native aorta and conduit. The amount of blood support received from either the native aorta or conduit depends on both the vessel geometry and the native aortic flow. Oscillatory flow patterns were observed on the inner curvature of the arch. This suggests that the addition of the conduit creates a low resistance outlet for blood to leave, causing native aortic blood flow rate to decrease and potentially results in higher vulnerability for intra-aortic plaques leading to thrombosis. CFD based on MRA and PCMR can be used to model hemodynamics in AVBS patients. Simulation results indicate that arch vessel perfusion is composed of both native aorta and conduit flow and depends highly on the native aortic flow. CFD results show regions of highly disturbed flow at the inner aortic arch, indicating a possible mechanism for post-surgical complications.

Computational studies on NapH1, a bacterial vanadium-dependent haloperoxidase

Amanda Li, Peter Bernhardt, Akimasa Miyanaga, Bradley S. Moore, Michael Gilson
University of California, San Diego

Marine microorganisms are a rich source of bioactive natural products that can be utilized in drug discovery and design. The enzymes responsible for synthesizing these natural products typically exhibit greater stereo- and regioselectivity than industrial catalysts, so the characterization and manipulation of natural product biosynthetic enzymes could advance the synthesis of novel chiral pharmaceutical agents. The vanadium-dependent haloperoxidase NapH1, found in Streptomyces sp. CNQ-525, catalyzes halonium-mediated meroterpenoid cyclization. The associated biosynthetic pathway generates antibiotic analogs of the napyradiomycin family. Computational modeling and simulation, as well as site-directed mutagenesis, were employed to characterize the binding action of NapH1 and its identified substrate SF2415B1. Molecular docking studies were used to evaluate putative binding sites and identify potentially functional residues as targets for mutagenesis. The substrate docked to the entrance site of a cavity proximal to the vanadate co-factor. Additionally, 3D models were generated for two homologous enzymes in the same biosynthetic gene cluster, NapH3 and NapH4 (with 51% and 68% sequence identity, respectively). These homology models were utilized to further characterize the molecular basis of substrate specificity. The combination of docking calculations and mutational studies provide insights and new hypotheses regarding the mechanism of this intriguing and potentially useful enzyme.

CellOrganizer: Image derived generative modeling

Devin Sullivan, Ivan Cao-Berg, Robert F. Murphy
Carnegie Mellon University; University of Pittsburgh

Simulating cellular behavior using computational models is a major goal of systems biology. Unfortunately, the ability to include sufficiently detailed information about the spatial organization of cells in such models is currently limited. CellOrganizer can provide such information by using a conditional generative modeling framework learned from live cell fluorescent imaging data. These conditional models allow for the simultaneous inclusion of a large number of proteins. Importantly this includes the ability to predictively model protein location and behavior based on the conditional structure of the models. Currently CellOrganizer can learn generative models for cell shape, nuclear shape, chromatin texture, vesicular organelle number, size, shape and position and microtubule distribution from two- or three-dimensional fluorescent imaging data. The statistical accuracy of these models are determined using a classifier trained on the 3D HeLa dataset and tested using generated images from models learned from this dataset. The accuracy of this classification is compared to classification accuracy of held-out real data.

Error Analysis and Correction of ADC Measurements for Gradient Non-Linearity

Dariya I Malyarenko, Brian D Ross, J. Brian Fowlkes, Thomas L. Chenevert
University of Michigan, Dept. of Radiology, MRI

Purpose: Apparent diffusion coefficient (ADC) measured by diffusion weighted imaging (DWI) has been suggested as a potential biomarker for cancer diagnosis and treatment monitoring [1]. Significant (>10-20%) spatial-dependent error in ADC measurement due to gradient non-linearity was demonstrated on commercial MRIs [2]. Our work seeks a practical procedure that both builds on comprehensive physical system characteristics [3-5] and achieves minimal algorithm complexity for quantitative control of experimental error.

Methods: Spatial dependence of gradient fields was modeled using spherical harmonic expansion to the 7th order [3]. Spatial dependence of b-matrix was calculated for three orthogonal DWI gradient directions along gradient coil axes ("lab"), as well as for an orthogonal combined axes scenario ("over-plus"). Gradient cross-terms were included through: (1) 3D-dependence of the non-linearity tensor [4], and (2) inclusion of imaging gradients [5] and their spatial dependence. Diffusion properties of the media were modeled using diffusion tensor with tissue-like characteristics: ADC =1.0 and FA = 0.0, 0.3, 0.5, 0.7 and 0.9. Tensor orientation was uniformly varied in respect to the lab (gradient) system. A correction was devised using only the leading terms of the spatial dependence of diagonal b-matrix elements. Assumed (uncorrected) ADC was obtained using b-values at the gradient iso-center where non-linearity is zero. ADC errors were calculated as deviation from true value for each pixel in 3D-volume within 30 cm FOV. Error statistics histograms were analyzed for ADC with and without b-correction.

Results: Contribution of gradient cross-terms to b-tensor was modest (<10 % of the diagonal values) in case of DWI gradients applied along the primary lab-axes, but were amplified for the over-plus. Increasing ADC non-uniformity errors were observed with higher anisotropy of the simulated medium. For isotropic case, the calculated errors were consistent with our experimental observations on clinical systems [2]. After leading b-term correction, residual error-distribution for ADC was dependent on anisotropic properties of the media and relative orientation of gradient fields. The absolute error reduction for ADC achieved through leading-term correction procedure was from 70 to 90% for anisotropic media (>95% for FA=0).

Conclusion: Spatial dependence of effective b-terms removes the bulk of ADC non-uniformity error. Residual error depends on FA of the medium and the DWI gradient direction/mode. ADC non-uniformity errors are amplified for anisotropic diffusion and gradient over-plus mode. Simplified b-correction algorithm, including spatial dependence of diagonal b-terms rotated into DWI gradient system, is found to be sufficient to control ADC error in clinical studies.

Identifying intermediate states within folding simulations

Andrej Savol, Chakra Chennubhotla
University of Pittsburgh

Purpose: The kinetic relationship between metastable conformational intermediates and protein folding rates has challenged both computational and experimental methods. The present paradigm recognizes a rough free energy landscape (FEL) with minima corresponding to stable states and conformational transitions as energetic barriers. However, exceptions to this simple abstraction are necessary for both intrinsically disordered proteins (IDPs), whose FELs are necessarily 'flatter', and also 'ultra-fast' folders, which lack significant energy barriers during folding. We investigate whether such metastable intermediates may exist in the case of the latter, specifically for the fast folding villin headpiece (VHP).

Methods: Long-run all-atom simulations are increasingly capable of accessing the timescales required to observe folding events. Our approach provides the needed subsequent analysis: what conformational states were accessed, how long were they visited, and which ones facilitated or inhibited transition to the native state? We studied long molecular dynamics simulations of VHP and identified non-native metastable states that function as waypoints within diverse folding trajectories. Rather than adopt structural similarity measures (RMSD, Rg) to probe structural transitions, we considered an embedded dihedral angle subspace where structural alignment bias is eliminated and overall dimensionality is drastically reduced. We additionally introduce temporal relationships within the trajectories by computing a general folding quotient and performing large scale clustering.

Results: As documented, more dihedral-based modes are necessary to capture an equal quantity of variance as its Cartesian-based counterpart, but these internal (dihedral) modes provided excellent sensitivity to near-folded intermediates and rarely visited states. We observed that commonly used folding order-parameters overlooked non-native contacts, even when such 'misfolding' is reproducibly necessary for achieving the eventual native state.

Conclusion: Taken together, our results address several organizing principles for the conformational landscape of fast-folders, and in particular for VHP. The approaches put forward here constitute a general framework for analyzing long folding simulations.

A multispecies continuum model of in vitro HGF-induced tumor spheroid growth.

Anna Konstorum, Stephanie Sprowl, Hamed Youssefpour, Dr. Arthur Lander, Dr. Marian Waterman, Dr. John Lowengrub
University of California, Irvine

There has been increasing evidence of the critical effects of microenvironmental influence on tumor growth and metastasis. Resident stromal myofibroblasts have been shown to induce B-Catenin nuclear localization and subsequent 'cancer stemness' in nearby tumor cells via secreted hepatocyte growth factor (HGF). The purpose of the current research is to develop an experimentally-validated mathematical model of tumor-host dynamics that includes both physical and chemical interactions of the two species. In collaboration with the Waterman laboratory in the Department of Microbiology and Molecular Genetics at UCI, we extend a multispecies continuum model of solid tumor growth developed in the Lowengrub laboratory to include data-validated HGF-induced effects on cellular proliferation and stem cell self-renewal capacity, and use the model to predict the effect of HGF concentration on the above parameters. Experimental methods include primary cell culture of colon cancer initiating stem cells in variable concentrations of HGF, and quantitative area measurement of tumor spheroids that develop in the cultures. The mathematical model is implemented using an adaptive finite difference-nonlinear multigrid method. Growth of tumor spheroids in control and +HGF conditions was successfully captured using our mathematical model, and sensitivity analysis on the parameters was performed to verify uniqueness of parameter selection. From the mathematical results for the control data, stem and transit-amplifying cell proliferation rates in a no-host environment were calculated and from the results for the +HGF data, the quantitative effect of HGF on stem and transit-amplifying cell division rate and probability of self-renewal was established. In conclusion, we have developed and experimentally validated a mathematical model of multispecies tumor growth in the presence of extracellular factors and used it to quantify the effect of the factors on growth parameters for the spheroids. This model will constitute a framework for a more extensive collaborative experimental / mathematical investigation into the dynamic nature of tumor-microenvironmental interactions.

Neural Engineering & Rehabilitation

The Hyperdirect Pathway as it Pertains to Side Effects Associated with Deep Brain Stimulation

Kabilar Gunalan, Angela M. Noecker, Kyle Taljan, Ken E. Sakaie, Cameron C. McIntyre
Case Western Reserve University

Introduction: Deep brain stimulation (DBS) alleviates the symptoms of Parkinson's disease but its mechanisms are not well understood. An emerging pathway of interest, thought to be modulated by DBS therapy, is the hyperdirect pathway that travels from cortex to the subthalamic nucleus (STN). Previous studies in rodents have shown antidromic activation of this pathway, resulting in modulated cortical activity. Furthermore, Frankemolle et al. (Brain, 2010) have noted cognitive-motor side effects in patients implanted with DBS electrodes using stimulation settings determined clinically (Clinical). These impairments improve by using settings that target the dorsal STN and neighboring white matter fibers (Model). In this study, we used tractography to understand which hyperdirect fiber pathways and cortical regions are associated with the cognitive side effects in the Frankemolle study.

Methods: A volume of tissue activated (VTA), estimated using the stimulation settings, was calculated for each of the 10 patients' Clinical and Model settings. The VTAs were mapped onto a common brain and, subsequently, diffusion-tensor-based probabilistic fiber tractography was performed using each VTA as a seed point. Results: Preliminary results show that the Clinical VTAs have more fibers terminating in frontal cortical regions than the Model VTAs.

Discussion: These fibers may account for the cognitive side effects associated with the Clinical settings, providing further evidence that modulation of the hyperdirect pathway is the mechanism by which DBS induces wide-scale network effects.

Conclusions: We used tractography to show that the cognitive side effects, induced by DBS, are associated with direct connections to frontal cortical regions.

Combining Dense Array EEG and Transcranial Magnetic Stimulation to Assess Cortical Reactivity and Co

Nessa Johnson, Bin He
University of Minnesota

Purpose: The purpose of this study was to combine navigated Transcranial Magnetic Stimulation (TMS) with dense array electroencephalography (EEG) to determine the dominant frequency and propagation of evoked activity resulting from TMS over motor, frontal, parietal, and occipital areas.

Methods: Navigated TMS was applied to motor, frontal, parietal, and occipital cortex during EEG recording in five healthy subjects. The neuronavigation system incorporated both anatomical and functional MRI data for each subject to ensure precise positioning of the TMS coil over the selected cortical regions. TMS was applied at motor threshold using a 70-mm figure eight coil connected to a Magstim Rapid2 stimulator. The EEG response was monitored using a TMS compatible 64 channel BrainProducts EEG system. TMS induced artifacts were removed offline from EEG data using both principle component analysis (PCA) and independent component analysis (ICA).

Results: The frequency content of the EEG response, resultant cortical current density distribution, and spatiotemporal propagation of TMS evoked activity differed depending on the stimulation site, but was similar across subjects.

Conclusions: The results suggest that the combination of MRI navigated TMS with EEG imaging provides sufficient spatiotemporal resolution to track the propagation of evoked activity over the cortex. When combined with EEG imaging, navigated TMS is an effective tool to assess the reactivity and connectivity among cortical areas.

Effect of global brain state on sensory processing by neurons in primary visual cortex

Yatsenko, D, Foudarakis, M, Saggau, P
Baylor College of Medicine

We study circuit-level mechanisms whereby populations of neurons of primary visual cortex encode visual stimuli. In the absence of external stimuli, populations of neurons remain spontaneously active. The patterns of spontaneous activity have been hypothesized to reflect the functional connectivity in the feedforward and recurrent circuits feeding into the neuronal populations. Spontaneous spike correlations as well as noise correlations during evoked activity are often analyzed as parameters determining the information carrying capacity of the network. However, the correlation structure of spontaneous activity is not static: shifts in the global cortical state or the behavioral state of the animal have been shown to alter it. Particularly well recognized is the distinction between activated cortical states and endogenously generated slow-wave activity (SWA) characteristic of some stages of sleep and anesthesia. In SWA, the brain is thought to be less attuned to afferent inputs than in information-processing activated states. How would SWA affect the structure of spontaneous population activity in the primary visual cortex and how would this change accounts for the change in the population response to external stimulation? To address these questions, we monitored the global brain state of adult mice under urethane anesthesia using local field potential recordings. Population activity of layer 2/3 neurons of the primary visual cortex was recorded using two-photon calcium fluorescence signals. Visual stimuli consisting of drifting gratings were used to measure the orientation selectivity of cells. In seven of the 11 mice analyzed, the cortical state alternated periodically between SWA and activated states with the period ranging between 1.5 and 6 minutes across mice while remaining stable across multiple recordings in the same animal. Responses to visual stimuli were significantly more reliable in activated states than in SWA partially due to lower baseline firing rates. The correlation structure of neuronal activity was significantly different between the two states: cortical activation triggered a significant decrease in pairwise correlations. Changes in the correlation structure were not accompanied by significant shifts in the tuning properties of cells. Additionally, visual stimulation decreased the pairwise correlations in both activated and slow-wave states. These observations are consistent with a model in which SWA is generated by endogenously in recurrent circuits with limited direct contribution to the receptive field properties of cells, that this activity is dissipated by both external stimuli and cortical activation, and that its input could be recognized and isolated by its multineuronal correlation structure.

Patterned sensory stimulation reduces urethral spasms and improves bladder voiding after spinal cord

Jaime L. McCoin, Kenneth J. Gustafson, Ph.D
Case Western Reserve University

Purpose: Damage to the central nervous system leads to muscle spasticity. For individuals with spinal cord injury (SCI), this spasticity can prevent bladder emptying and cause medical complications. Overactive reflex contractions of the external urethral sphincter (EUS) are not well controlled by medication and may require destructive surgery. We have developed a novel technique that uses electrical sensory stimulation to reduce these EUS spasms by modulating spinal cord reflexes. Our goal is to demonstrate that sensory stimulation can improve bladder voiding and function as a long-term neuroprosthesis after SCI.

Methods: Sensory stimulation was applied to the sacral skin of eight adult male cats to evaluate which stimulus patterns and locations could suppress urethral reflex activity. Nerve cuff electrodes were implanted on the extradural S2 sacral roots to generate sufficient bladder drive for voiding. The spinal cord was transected at T10/T12. Post-SCI testing showed the progressive development of abnormal urethral reflexes, including reflexes interrupting voiding. In two animals that demonstrated suppression, electrical voiding was applied 2-4x daily to replace hand expression or catheterization. Control voids without afferent stimulation were randomized across the treatments. Sensory stimulation consisted of (.75 ON, .25 OFF 20 Hz) or 20 Hz continuous patterns. Stimulation was repeated with 3 minutes of rest in between to maximize emptying.

Results: Sensory stimulation worked to suppress reflexes in four of the eight animals. Surface locations generating significant suppression were discovered in 2 animals 3 and 4 weeks following SCI, respectively. These animals demonstrated significant improvements in emptied volumes when compared with voiding without sacral dermatome stimulation. One animal was maintained using only electronic stimulation for 5.5 consecutive days; the second animal was maintained for at least three days per week for 3 consecutive weeks.

Conclusions: Stimulating sensory fibers can suppress these urethral reflexes in animals with chronic spinal cord injury. We have translated this technique into a clinically effective method of bladder maintenance in awake animals. This is the first step towards a non-invasive neuroprosthesis for voiding, and we have begun testing in human subjects with spinal cord injuries.

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Neuronal oscillatory correlates associated with working memory performance

Heister D, Diwakar M, Nichols S, Robb A, Angeles A, Tal, O, Harrington, D, Lee, RR, Huang, MX

University of California, San Diego

Purpose: Working memory represents the brain's ability to maintain information in a readily available and malleable state for short periods of time. Currently, it is not clear what electro-magnetic brain activities and corresponding neuronal substrates are most associated with working memory performance. The current study used magnetoencephalography (MEG) to investigate the regional patterns of cortical activity that most correlated with performance during a difficult verbal working memory task.

Methods: The neural patterns generated by a population of adult men (18-30 yrs, n=24) were collected in both a resting state and during 3 progressively more challenging verbal N-back working memory tasks. Changes in sensor- based regional power across conditions, and changes in inter-regional correlations for band- passed signals (theta/delta (3-8 Hz), alpha (8-13 Hz), beta (13-30 Hz)) were analyzed. The most dominant mode obtained from the Singular Value decomposition (SVD) of normalized data was then used to determine the degree to which changes in regional band-passed power and inter-regional correlations were associated with performance on the 3-back verbal working memory task.

Results: During the resting-state, increases in frontal cortex weighted 3-8 Hz frequency power was correlated with 3-back working memory d prime measured performance ($r=0.64$, $p<.001$). This association persisted when subjects were actually performing the working memory task ($r>.43$, $p<.05$; for all 3 conditions). Additionally, inter-regional increases in correlations at (8-13Hz, and 13-30Hz) weighted largely in the bilateral parietal and occipital regions during the N-back task also correlated with better 3-back working memory performance (8-13 Hz: $r=.55$ $p<.01$; 13-30 Hz :$r=.50$, $p=.01$). Lastly, an SVD was performed on all interregional correlation values ($p<0.01$ threshold) to compute a composite measure for each subject that characterized multiple-band and regional comparisons during N-back performance. This composite measure highly correlated with 3-back working memory performance($r=.63$, $p<.001$).

Conclusion: Our preliminary results indicate that there is an association between increases in frontal 3-8Hz neural-oscillatory power both before and during the N-back task and working memory performance. Moreover, Nback task related increases in inter-regional alpha and beta correlations weighted in the parietal and occipital cortices are also associated with working memory performance, and a composite measure of regional neural oscillatory correlations further improved this association. To our knowledge, this is the first investigation that has non-invasively isolated and distinguished the neural patterns associated with verbal working memory performance in humans during both the resting state and the working memory task.

An Optogenetic Micro-Electrocorticography Neural Interface

Thomas Richner, Sanitta Thongpang, Sarah Brodnick, Lisa Krugner-Higby, Justin C. Williams

University of Wisconsin

Purpose: Neuroprostheses have the potential to help individuals who have lost use of a limb. However, previous micro-scale neural interfaces have short useful lifetimes due to glial scarring. Micro-electrocorticography (micro-ECoG) arrays, which rest on the surface of the cortex, are somewhat less invasive. However the spatial and temporal limits of micro-ECoG have not been well described and are difficult to address accurately by conventional electrophysiology. Optogenics (light activated ion channel transgenes expressed in neurons) enables direct stimulation of multiple spatially and temporally defined populations of neurons. We leveraged optogenetics to test micro-ECoG array designs and study cortical dynamics.

Methods: Micro-ECoG electrode arrays (16 platinum sites, 200 um diameter, 500 um spacing, Parylene C) were fabricated and implanted onto the cortical surface of Thy1-ChR2/H134R mice under a cortical window using sterile technique. All animal procedures were approved by the UW IACUC. Blue light was applied through the cortical under sedation (ketamine & dexmedetomidine) while micro-ECoG signals were simultaneously recorded. LEDs and LASERs were either broadly applied or focally applied (200 um diameter region) with a microscope. Fibers were also used in terminal experiments determine the contribution of different cortical layers to the micro-ECoG signal.

Results: Broad LED stimulation with brief flashes of blue light (<10 ms) evoked large (up to 1.5 mV) negative potentials that were dependent on pulse duration and amplitude, and focal stimulation evoked spatially localized evoked amplitudes that fell off by more than 80% over 1.5 mm. Spatial and temporal resolution was quantified with pairs of pulses, and the frequency response was found to match the limits of the ChR2/H134R channel. The interface was stable over multiple weeks. The photoelectric effect was orders of magnitude smaller (> 100x) than the optogenetic potential when measured in vivo after pentobarbital overdose, and yellow light did not evoke an appreciable ChR2 response. Fiber stimulation evoked layer-specific micro-ECoG signal patterns with increased inhibitory (positive) peaks with stimuli at layer 2/3 and larger diffuse excitatory (negative) peaks when layer 5 was stimulated. Longer light pulses (50 ms) at layer 2/3 caused transient gamma bursts that were dependent on interstimulus interval and pulse amplitude, and stimuli during or shortly after spontaneous gamma bursts had reduced amplitude suggesting transiently increased feedforward inhibition.

Conclusions: Building upon this work, future iterations will include projector technologies, varied electrode designs, implanted waveguides and integrated LEDs. Network analysis, closed-loop stimulation and source localization algorithms are also new directions.

Characterization and detection of walking-stair transitions in able-bodied ambulation

Joshua Peng, Levi Hargrove
Northwestern University

Purpose: The objective of this study is to characterize 'walking-stair' (level walking to stair ascent/descent) transitions in able-bodied ambulation. The initial portion of this study will be to characterize lower limb electromyography (EMG), temporal-spatial parameters, kinematics, and kinetics before and during the 'walking-stair' transitions in order to detect transitions that are imminent or occurring. Characterization and detection of walking-stair transitions in able-bodied ambulation is a crucial step to implementing seamless transitions in the control system of a powered transfemoral prosthesis. Previous studies and preliminary data have shown that able-bodied subjects significantly adjust muscle activity, motion of the limbs, and/or forces applied between the feet and ground during mode (e.g. level walking, ramp ascent, stair ascent) transitions; however there has not been a full investigation of anticipatory responses to walking-stair transitions.

Methods: We will recruit 10 able-bodied subjects to approach and transition onto a 4-step staircase during which we will acquire kinematic and temporal-spatial data from motion capture, kinetics from embedded force plates in the floor and stairs, and surface EMG from 12 lower limb muscles (both legs). We will identify the most significant changes to employ as markers to detect when a user is intending to perform a walking-stair transition. Our central hypothesis is that EMG will allow us to detect walking-stair transitions significantly earlier than with just kinematic and kinetic data. We expect significant changes in EMG activation patterns during stair transition strides will occur before toe off of the transitioning leg.

Results: Preliminary data indicates that EMG may allow sufficiently early transition detection before this critical time to signal a change in the prosthesis settings to assist the user in the transition.

Conclusion: Following transition detection, future work entails developing a controller for a powered, impedance-controlled transfemoral knee prosthesis that can appropriately adjust the mechanical properties at the knee and ankle to provide continuous, automatic, and more natural transitions between ambulation modes.

Biomechanics

The intensity-dependent release of triggered reactions modulates the long-latency stretch reflex

Rosalind L. Heckman, Claire F. Honeycutt, Eric J. Perreault
Northwestern University

The long-latency stretch reflex (LLR) is adaptable like voluntary movements, yet occurs at reflex latencies. It contains at least two components that can modulate in a task-appropriate manner: one opposing muscle stretch and another associated with the rapid release of planned movements. The component of the LLR related to the release of planned movements has been described as a "triggered reaction," which should remain invariant with respect to perturbation amplitude. However, it was recently shown that this component can scale with perturbation intensity, arguing against triggered reactions, and the proposed mechanisms for their release. The mechanisms proposed for triggered reactions suggest a functional contribution following stroke, if we can resolve their existence with the observed amplitude scaling of the LLR. A possible explanation is that the probability of releasing a triggered reaction varies with perturbation intensity, as is the case for auditory-triggered reactions. The purpose of our study was to evaluate two hypotheses regarding the amplitude scaling of the LLR: 1) amplitude scaling arises from a feedback response to the imposed muscle stretch; and 2) amplitude scaling arises from an intensity-dependent probability of releasing a triggered reaction, appearing as amplitude scaling when multiple responses are averaged. Data were collected from 13 subjects instructed to make ballistic elbow extension movements. Flexion perturbations ranging from 0.5-120°/s were applied with the cue to initiate movement. Activation of the sternocleidomastoid (SCM) neck muscles was used to indicate the presence of a startle-like response, which we have shown corresponds to the release of a triggered reaction. Twenty repetitions were collected for each perturbation, presented in a block-randomized order. LLRs from the lateral head of the triceps muscle were quantified as the average EMG amplitude between 75-105 ms after the perturbation. We found that the probability of releasing a triggered reaction increased with perturbation velocity. LLR amplitude was larger for reflexes elicited in the presence of SCM activity for all perturbation velocities. These results accounted for some amplitude scaling observed when considering average responses. However, there was still scaling of the LLR when considering trials without SCM activity. This is likely attributed to the amplitude dependence of the LLR elicited in the absence of a planned movement. In support of both hypotheses, scaling of the LLR with perturbation intensity appears to result from an intensity-dependent probability of releasing a triggered reaction when a movement has been planned, and an intensity-dependent feedback response to the perturbation.

Impact of Parity on the Mechanical Properties of the Sheep Vagina

Katrina Knight, BS, Andrew Feola, PhD, Pamela Moalli, MD, PhD, Steven Abramowitch, PhD

University of Pittsburgh

Introduction: Pelvic organ prolapse (POP) is defined as the descent of pelvic organs into the vaginal canal with parity (number of births) being the leading risk factor for the development of POP. Parity negatively impacts the vaginal mechanical properties of the non-human primate (NHP). Rats, however, rarely sustain birth injury and generally do not develop POP. Our future goal is to utilize a tissue engineering approach to obviate the decline in vaginal mechanical properties secondary to birth injury. Thus, the purpose of this study was to determine the impact of parity on the mechanical properties of the sheep vagina, a large animal model that is cheaper than the NHP and known to develop POP naturally.

Methods: Uniaxial tensile tests were performed on seven nulliparous and eight parous sheep vaginas. Samples were harvested from the mid-posterior vagina and cut into a dog-bone shape. Cross-sectional area was measured using a laser micrometer and strain was measured optically via surface markers. Samples were rigidly fixed in soft-tissue clamps attached to an InstronTM testing machine while submerged in a physiological saline bath. A preload of 0.1 N was applied followed by 10 cycles of preconditioning to 7% of the initial clamp-to-clamp distance and lastly samples were loaded to failure at a rate of 10 mm/min. The tangent modulus, tensile strength, ultimate strain, and strain energy density were determined. Comparisons were made using an unpaired student t-test ($p<0.05$).

Results: There was no difference in the tangent modulus between the nulliparous and parous vagina, 19.8 ± 9.2 and 21.1 ± 5.3 MPa respectively, $p = .74$; nor was there a difference in the tensile strength, 2.76 ± 0.95 and 2.45 ± 0.90 MPa respectively, $p = .54$. Additionally, the ultimate strain for the nulliparous and parous vaginas were not different, 31.0 ± 11.0 and 23.0 ± 6.0 % respectively, $p = .10$; nor was the strain energy density, 0.32 ± 0.14 and 0.22 ± 0.13 MPa respectively, $p = .15$.

Conclusions: Unlike primates, parity had little effect on the mechanical properties of the sheep vagina despite nulliparous sheep, rat, and NHP vaginas having similar mechanical properties. Thus, the development of POP in sheep may be a consequence of something other than trauma to the vagina during delivery, e.g. tail docking. Future studies will utilize a simulated birth injury in a rat to determine if it is a suitable model for testing a tissue engineering treatment approach.

Biomedical Devices / Platforms

Biomechanical Evaluation of Bioabsorbable Polymer Interference Screws for ACL Reconstruction

K.E. Kim, M.M. Tei, A.N. Pickering, K.F. Farraro, S.L-Y. Woo
University of Pittsburgh

PURPOSE: Due to the permanency of metallic interference screws (e.g. titanium) for ACL reconstruction, an increasing number of bioabsorbable polymer screws are used as they were intended to biodegrade and let the bone-tendon graft interface to regenerate. However, these devices also suffer from inconsistent or minimal degradation, in addition to screw breakage and poor osteointegration. In this study, we assessed fixation of polymer screws in terms of graft slippage and fixation strength first at time zero and then at 12 weeks of healing in a goat model.

METHODS: For the time zero study, a BPTB ACL reconstruction was performed on 4 cadaveric goat stifle joints using a polymer screw, which was provided by Depuy-Mitek gratis. Titanium screws were used as a control. For the in vivo surgery, a bone-patellar tendon-bone ACL reconstruction was performed on 6 Spanish breed goats using the polymer screws. At time zero, three cyclic loading tests were performed to measure graft slippage (unrecoverable elongation of the femur-graft-tibia complex (FGTC)) and a load-to-failure test for the ultimate load of the FGTC. After 12 weeks, the animals were euthanized, and the harvested joints were tested on a robotic testing system for joint stability, followed by a load-to-failure test for the ultimate load of the FGTC.

RESULTS: At time zero, the total graft slippage was 1.9 ± 1.1 mm and 1.4 ± 0.4 mm for the bioabsorbable and Ti screws, respectively ($p > 0.05$). The ultimate load values of the FGTCs were 235 ± 71 N and 234 ± 13 N respectively ($p > 0.05$). After 12 weeks of healing, the in situ forces in the tendon graft were 20 ± 16 N, 8 ± 3 N, and 12 ± 11 N at 30°, 60°, and 90° flexion respectively. The ultimate load of the FGTCs reconstructed with bioabsorbable screws was 156 ± 42 N.

CONCLUSIONS: The amount of graft slippage at the time of screw placement was comparable to the titanium screws, as was the ultimate load of the FGTC. However, after 12 weeks, mechanical function of the graft affixed by the polymer screws deteriorated as the ultimate load of the FGTC became significantly lower compared to that at time zero. Also, in-situ forces of the graft were lower than those from comparable animal models [5,6] . These findings suggest loss of adequate fixation over time, which may be due to both graft slippage and poor graft-tunnel healing.

A Sound-to-Touch Sensory Substitution Device for the Severely Hearing Impaired

Scott Novich, David M. Eagleman
Rice University; Baylor College of Medicine

There are at least 2 million functionally deaf individuals in the United States alone and an estimated 53 million worldwide. The cochlear implant (CI) is an effective solution for regaining hearing capabilities for certain populations within this group, but not for all. First, CIs are expensive, ranging from $40,000 to $90,000 depending on the age of the recipient. This places CIs out of economic reach for many. Second, CIs require invasive surgery. Third, there is low efficacy of CI implantation in early-onset deaf adults over the age of 12. Given this, there exists an important population of deaf individuals who would benefit from a hearing replacement that has low cost, does not involve an invasive procedure, and may have a higher efficacy for early-deaf adults over the age of 12. To address these problems, we are developing a low-cost, non-invasive, plasticity-based solution to deliver auditory information to the brain. Specifically, we are developing a "vibratory vest" by which auditory information is captured, digitally processed, and delivered to the skin of the torso using small vibratory motors. We call our device the Vibrotactile Acoustic Coder, or the "VAC." The term for such a technique is sensory substitution, and has previously proven successful in allowing those who are blind to have visual experience through the tongue or skin. Although auditory sensory substitution systems (known as "tactile hearing aids") have been prototyped as early as the 1970s (and proposed as early as the 1920s), a successful project is only now made possible by recent strides in digital signal processing techniques, speech codecs, machine learning, and processor power and cost. Our device leverages all of these advances to develop tactile encoding algorithms that extend beyond the simple filtering and mapping of an incoming audio stream as found in previous tactile aids. Our device will cost an order of magnitude less than a CI, be non-invasive, and ?we hypothesize? not require critical period plasticity for use. From a broader perspective, this endeavor will enable us to better understand and model how the brain processes any sort of sensory information, understand the properties and capabilities of the brain's plasticity, and help us develop a generic framework for designing effective sensory-substitution devices. In this poster, we will present the current development status of the VAC and preliminary results of a haptic time perception experiment.

Phase Aliasing Enhancement to DUET Blind Speech Separation Algorithm

Ryan Ritch, Jack Xin
University of California, Irvine

A significant problem plaguing users of hearing aids and cochlear implants is following conversations in the presence of interference such as in a noisy room. One area of active research that seeks to address this issue is the so-called cocktail party or Blind Speech Separation (BSS) problem. In this setting, multiple audio sources (such as voices) at distinct physical locations are recorded using several microphones with the goal being recovery of the individual sources. We investigated one technique for solving this problem known as Degenerate Unmixing Estimation Technique (DUET). This method uses clustering and the assumption that different audio sources occupy different locations in frequency space to separate the mixes into their constituent components. Additionally, it has the advantage of being able to solve the underdetermined case of more sound sources than microphones. However, one of the method's shortcomings is the necessary of placing the microphones extremely close to each other to avoid phase aliasing, reducing the overall efficacy of the segmentation. In our research, we work to overcome this limitation by implementing a novel phase aliasing correction algorithm. This modification calculates difference in phase between sources much more robustly, enabling enhanced source localization and hence superior separation. Additionally, we investigate using fuzzy clustering techniques to improve subjective separation quality when conditions such as reverberation render the standard DUET algorithm ineffective. Our enhanced algorithm provides noticeable gains in segmentation performance over the unmodified DUET algorithm, inviting the possibility of substantially improving the quality of life of users of assistive devices.

A System for Real Time Visualization of Platelet Deposition onto Opaque Surfaces

Megan A. Jamiolkowski, Joshua R. Woolley, Sang-Ho Ye, Salim Olia, Marina Kameneva, James F. Antaki, William R. Wagner
University of Pittsburgh

Purpose: Due to erythrocyte opacity, it has been difficult to develop a method for assessing real time platelet deposition onto opaque surfaces using whole blood. Using hemoglobin depleted red blood cells (RBC ghosts) and long working distance optics we sought to develop a system for such visualization.

Methods: The application of RBC ghosts as a blood model was validated by comparing the kinematic viscosity and deformability of the RBC ghosts to native red blood cells (RBCs). Fluorescently labeled platelets were mixed with RBC ghosts at a hematocrit of 25% and perfused through a custom designed parallel plate flow chamber across a variety of clinically relevant opaque biomaterial surfaces.

Results: Fluorescent images of platelet deposition were acquired in real time and were analyzed for platelet surface coverage using a customized Mat Lab (Math works) program. Platelet deposition was verified by scanning electron microscopy. The kinematic viscosity and deformability data of the RBC ghosts and native RBCs was analyzed using a repeated measure ANOVA, with no significant differences detected ($P > 0.05$, $n=9$).

Conclusion: The results suggest that this is a viable method for assessing the acute hemocompatibility of opaque materials. Many manufacturers of ventricular assist devices (VADs) use a highly polished titanium alloy, TiAl6V4, as a blood contacting surface. However the utilization of alternative materials/coatings could improve the biocompatibility of the device. The described system could be a useful tool for comparative analysis of candidate VAD blood-contacting materials.

Micro-scale Optofluidic Ring Resonator Sensors for Micro Gas Chromatographs

Kee Scholten, Xudong Fan, Edward Zellers
University of Michigan

Purpose: The need for in-situ determinations of volatile organic compounds (VOCs) for applications including environmental analysis and diagnostic biomarker evaluations of breath and saliva continues to drive the creation of microfluidic components for micro gas chromatographs (µGC). Considerable work has demonstrated optical resonators as small yet sensitive transducers; minute shifts in resonant frequencies of waveguide structures indicate capture or interaction of an analyte with a sensitive chemical coating. Here we describe progress toward microfabricated VOC sensors comprising novel optofluidic ring resonator (µOFRR) structures integrated with on-chip microfluidics and selective chemical interfaces of plasmonic, nanoparticle films.

Methods: Conventional microfabrication techniques were used to create partially released, hollow silica structures (diameter 50-200 µm, wall thickness < 2 µm) with toroidal expansions that serve as an optical ring resonator. A combination of iso- and anisotropic plasma etches were used to create a Si mold defining the structure; subsequently a series of thermal oxidations were used to smooth the walls of the device before growing the structural oxide. In on-going efforts we are fabricating plasma-etched microfluidic interconnection channels and fiber optic alignment structures to create a lab-on-chip platform to facilitate the sensing applications of these resonators. Additionally we are exploring thiolate-monolayer protected gold nanoparticle films as chemical interfaces in the hopes of obtaining discriminatory multi-wavelength responses to vapor sorption. Films of octanethiol capped gold nanoparticles on planar Si substrates were excited by lasers at two visible wavelengths (783 nm and 488 nm) and reflected light was measured during exposure to VOCs.

Results: Whispering gallery modes were excited in the walls of the µOFRRs by evanescently coupling light from tapered optical fibers. Measured quality factors exceeded 104, and measurements of free spectral range confirmed the presence of circulating modes. Characterization of µOFRRs as VOC sensors is ongoing. Nanoparticle films were successfully demonstrated as a selective interface for such optical transducers. Responses were linear with injected analyte mass and reversible under flow. The wavelength dependent sensitivity to tested VOCs (heptane and toluene) yielded distinct and identifiable relative responses.

Conclusions: Novel devices capable of producing on-chip optofluidic detectors have been fabricated and characterized. Nanoparticle films have been successfully demonstrated as optically sensitive interface layers for VOC transduction, and a novel method of increasing the dimensionality of VOC discrimination has been established. Future efforts will entail coating arrays of optofluidic ring resonators with differently functionalized nanoparticle films, and integration with existing µGC platforms for analyzing complex VOC mixtures.

Moire Wavefront Sensor as an Alternative to the Shack-Hartmann for Opthalmic Applications.

Carl Chancy
University of Arizona College of Optical Sciences

Purpose: The Shack-Hartmann wavefront sensor is key technique for the development of adaptive optics technology in astronomy. In a Shack-Hartmann sensor, the collimated wavefront is imaged onto a lenslet array which form spot pattern at the rear focal length of each lenslet. The deviation of each spot can be can be used to reconstruct the wavefront and determine the aberrations present. The same technique has been used in ophthalmic purpose to measure aberrations in order to improve vision. However, due to the size and separation of the lenslets there are inherent limitations in dynamic range and sensitivity when measuring unusually shaped cornea. An alternative method of wavefront sensing uses Talbot imaging or self-imaging to reconstruct the incident wavefront. When a grating is illuminated by coherent light, it is imaged at speci?c locations behind the grating called Talbot distances. Placing a second grating at one of the Talbot planes will generate a moiré pattern. Similarly, when an aberrated wavefront is incident onto the gratings, the images at the Talbot planes are encoded with all of the phase information and the moiré pattern can be used to reconstruction the wavefont. Our goal is to compare the performance of the Moire Sensor to the Shack-Hartmann wavefront in order to determine its dynamic range, sensitivity and reliability.

Method: A 532nm laser is used to illuminate a 50 actuator deformable mirror. The deformable mirror is used to generate specific amount of defocus, astigmatism, spherical aberration and coma. A beam splitter is used to divide the wavefront so that simultaneous measurements can be made with the Shack-Hartmann and Moire wavefront sensor. The spot patterns from are Shack-Hartmann are reconstructed and ?t to Zernike polynomials. The Moire patterns are reconstructed using a Fourier transform technique where the phase information in the x and y direction can be retrieved separately. The Zernike polynomials are then compared to the theoretical values.

Results and Conclusions: Preliminary results have shown that the moiré wavefront sensor can accurately measure large amount of defocus, astigmatism, spherical aberration and coma. The next phase is to generate more complex surface pro?les and determine the sensitivity and reliability of moiré wavefront sensor. In addition, the reconstruction software needs to be improved so the ?lters can automatically select the appropriate region of interest in order to reduce noise and increase repeatability.

User-friendly Hydrodynamic Single Cell Capture Devices for Cancer Stem Cell Screening

Patrick Ingram, Jaehoon Chung, Kun Yang, Ronald J Buckanovich, Euisik Yoon
University of Michigan; Dept of Biomedical Engineering

The cancer stem cell (CSC) or tumor initiating cell (TIC) model proposes that a small subset of stem-like cells necessary to sustain cancer growth. CSC are resistant to traditional therapy and capable of asymmetric division to give rise to a heterogeneous population. Therapeutics which target CSC have drastically improved patient survival. There are, however, obstacles to the study of CSC: 1) CSC are rare, representing typically <5% of cells in cell lines and <1% of cells in tumors, 2) CSC are a 'moving target', rapidly undergoing asymmetric division, such that pure CSC populations are transient and difficult to study over time, and 3) CSC are difficult to identify and grow. Furthermore, large scale screens for therapeutics capable of specifically targeting CSC are challenging as traditional screening methods typically focus on reduction of overall tumor cell number to evaluate success of cancer treatment. Thus, therapies eliminating the rare, but critical, CSC would be missed in traditional screens. This may account for limited improvement in cancer morbidity and mortality. There is a clear need to provide single cell tools to expedite the characterization of CSC and the development of CSC based therapies. While many tools, both microfluidic and others, have been developed to address some of these issues, they are rarely deployed outside of the labs that design them due to considerable overhead, external equipment requirements, and complex operation. To be widely applied in laboratory and clinical settings, new single cell tools must be simple to operate and mesh well with current laboratory practice. In this respect, we have developed a microfluidic platform that can handle and culture (in both adherent and non-adherent environments) single cells in high throughput, allowing faster analysis of the heterogeneous population of cancer cells, specifically CSC, with a micropipette as the only external tool. This system has achieved high efficiency single cell capture (>90%). In this way, heterogenous populations have been captured and cultured on extracellular matrix coated glass surfaces in the wells. In addition by introducing anti-biofouling, hydrophobic surfaces, the wells have been modified for non-adherent spheroid culture. The platform is highly modular and has been used to investigate prostate cancer (PC3) drug resistance in clonal culture and single breast cancer (MCF7 and SUM159) CSC derived spheroids among others. Our approach is attractive in terms of its simplicity and low-expertise requirement, and can expedite phenotypic analysis of rare and heterogeneous cell types.

Quantitative testing of robust dry reagent storage with filter paper

Grace Wu, Jaya Srivastava, Muhammad Zaman
Boston University

PURPOSE: Several paper-based devices have demonstrated the ability to detect biological disease markers with high sensitivity and specificity. Paper itself is low-cost, light-weight, and disposable, making it an ideal platform for point-of-care health testing in the developing world. However, recent literature lacks in quantitative information on the stability of protein reagents stored on paper-devices, which can affect device performance and determine its robustness in the field. In our study, we quantitatively characterized the functionality of proteins stored on filter paper under conditions that mimic those found in resource-limited settings. Furthermore, we explored methods to improve filter paper's efficacy on storing reagents.

METHODS: Protein reagents were spotted on filter paper (~1 cm in diameter) or glass substrates, dried, and stored under different conditions for predetermined durations. The protein reagent was FITC-conjugated anti-beta integrin antibody, an antibody against a common cell surface marker. After storage, the antibody was reconstituted from the filter paper, incubated with prostate cancer cells, and analyzed by flow cytometry to measure the mean fluorescence intensity (MFI). Cells incubated with or without non-filter paper stored antibodies served as positive and negative controls, respectively.

RESULTS: Protein reagents stored on filter paper retained functionality, but exhibited a non-linear decay with time and elevated temperature. Between one and two weeks, proteins stored on filter paper at 25*C resulted in 40% and 70% decreases in MFI. At 37*C storage, the MFI decrease was even more drastic. However, at both temperatures, proteins stored on filter paper performed better than those stored on glass substrates. Improvement in MFI, at all temperatures and durations, could be seen if the reagents were stored with 5% trehalose, a known preservative. Higher concentrations of trehalose did not significantly improve MFI. Furthermore, MFI recovery was 35% higher when filter paper samples were stored in the presence of silica beads. Pre-soaking filter paper in sodium hydroxide, a common treatment in the clothing industry, did not improve MFI.

CONCLUSIONS: Filter paper stabilizes proteins during storage in harsh environmental conditions, despite some activity loss. We have identified methods that can mitigate such losses (e.g. storage with silica beads and trehalose), which can be applied to paper-based immunological tests targeted for resource-limited settings. Overall, this research has helped improve our understanding of the mechanism of interaction between proteins and filter paper. Incorporating the results of this research into existing paper-based devices can make point-of-care tests significantly more robust, reliable, and affordable.

A Paper and Plastic Device for Performing Recombinase-Polymerase Amplification of HIV DNA

Brittany A. Rohrman, Rebecca R. Richards-Kortum
Rice University

Purpose: Despite the importance of early diagnosis and treatment of HIV, many HIV-exposed infants in low- and middle-income countries are not tested for the disease. The gold standard for early infant diagnosis, DNA PCR, requires resources that are unavailable in poor settings, and no point-of-care HIV DNA test is currently available. Several technologies for point-of-care sample preparation and nucleic acid detection have been developed using paper-based microfluidics, but enzymatic amplification in paper has not yet been demonstrated. The purpose of this research is to develop a paper and plastic device that performs isothermal, enzymatic amplification of HIV DNA.

Methods: We first optimized a protocol for performing recombinase polymerase amplification (RPA) in solution to amplify HIV DNA. Then several types of materials were screened for their ability to support RPA in a matrix-based format. Finally, we developed a device constructed of laser-cut layers of paper, glass fiber, adhesive, and plastic that is assembled by stacking components. To operate the device, the user must pipette reagents onto pads contained in the device, dip part of the device into the sample containing DNA, and fold components of the device together. We used commercially available lateral flow strips to demonstrate the successful amplification of HIV DNA.

Results: We showed that RPA can be performed successfully in solution, in several types of materials, and in RPA devices. Our device can amplify 10 copies of HIV DNA to detectable levels in 15 minutes while retaining many attributes desired for a point-of-care test. The device is small, light-weight, easy to assemble, and requires only five steps for operation. The only laboratory infrastructure required consists of a micropipette, pipette tips, and a heater.

Conclusion: Our results suggest that the RPA device, which is designed to be used after DNA extraction from dried-blood spots and in conjunction detection on lateral flow strips, may serve as part of an HIV DNA test to be used at the point-of-care in low-resource settings.

Elucidating genes and pathways in lipid storage and distribution in C. elegans by novel microfluidic

Maria Elena Casas, Hang Lu
Georgia Institute of Technology

Purpose: Obesity is a serious health issue that is contributing to the increasing rate of diseases affecting two thirds of Americans [1]. The risk associated with obesity is linked to fat distribution in the body which has a genetic component that is yet to be fully understood. C. elegans is a convenient model because analysis of key lipid storage and metabolic pathways can give insight into the pathways in humans [2]. Lipid droplet expansion in C. elegans has been linked to dysfunction in the peroxisomal pathway, where the gene dhs-28 encodes for enzymes responsible for processing fatty acid; similarly, the human orthologue controls catabolism of straight-chain and branched-chain fatty acids. [3]

Methods: Current methods for quantitative analysis of C. elegans require time consuming microscopy. Engineering techniques to increase throughput and improve objective quantification would dramatically enhance the ability to study the lipid distribution and pathways in C. elegans. Microfluidics advances this field through micro scale fluid flow manipulation for precise control of the organism. Automated image processing along with microfluidic devices, were developed to obtain higher throughput and quantitative results, allowing for robust conclusions.

Results: In imaging dhs-28 mutants through microfluidic devices, and altering the E.coli food source, it was possible to observe that lipid droplet size and distribution is dynamic and is tied to storage and peroxisomal catabolism of fat.

Conclusion: Future experiments would include feeding C. elegans different genetically altered E.coli to determine protein function in lipid storage. This research might ultimately help reduce the high occurrence of obesity in humans and lead to treatments of diseases such as diabetes.

[1] S. Gesta et al., Cell, vol. 131, pp242 – 256, 2007. [2] B. Mullaney and K. Ashrafi., Biochimica et Biophysica Acta, vol. 1791, pp 474-478, 2009. [3] S. Zhang et al., PNAS, vol. 107, pp 4640-4645, 2010

Microfluidic technology for the isolation of pathogenic bacteria in bloodstream infections

Lorenzo D'Amico, Thomas E. Anderson, Javier A. Adachi, Peter R.C. Gascoyne
University of Texas at Austin

Bloodstream infections are a major cause of death in immunocompromised, neonatal and elderly populations worldwide with approximately 50% mortality rate. Early detection, when there is less that 1 bacterium per mL of peripheral blood, greatly improves clinical outcome yet represents an enormous challenge involving highly trained personnel, significant expense and lab infrastructure. These resources are not accessible to the great majority of the world's population. Biochip devices can improve clinical outcomes, increase access to care and reduce associated costs by providing less expensive, automated, portable, and more sensitive diagnostic tools. However many chip-based bioseparators and biosensors reported in the literature are limited by the need for external instrumentation. In this study, an instrument capable of performing label-free, antibody-independent pathogen capture and detection is being developed as a proof-of-concept tool to aid in the diagnosis of bloodstream infections. Saponin lysis, which is a standard step in current culture methods for detecting septicemia, is used to permeabilize and kill the human blood cells while leaving bacteria viable. A membraneless microfluidic dialyzer is then used to reduce the conductivity of the specimen. This allows the intrinsic dielectric properties of the pathogenic microorganisms present in the sample to be exploited and for these cells to be trapped on a microelectrode array surface by dielectrophoresis without the need for biochemical labels and bioengineered tags. The target microorganisms are thereby isolated from the blood cell debris and plasma and concentrated within a microchamber. The eventual goal will be to culture the trapped bacteria in the microchamber and incorporate electrochemical impedance spectroscopy as the means of detection.

Tailoring the Trajectory of Cell Rolling with Cytotactic Surfaces

Collin D. Edington, Hironobu Murata, Richard Koepsel, Jill Andersen, Sungeun Eom, Takeo Kanade, Anna C. Balazs, German Kolmakov, Carsen Kline, Daniel McKeel, Zvi Liron, Alan J. Russell
University of Pittsburgh

Cell separation technology is a key tool for biological studies and medical diagnostics that relies primarily on chemical labeling to identify particular phenotypes. An emergent method of sorting cells based on differential rolling on chemically patterned substrates holds potential benefits over existing technologies, but the underlying mechanisms being exploited are not well characterized. In order to better understand cell rolling on complex surfaces, a micro- fluidic device with chemically patterned stripes of the cell adhesion molecule P-selectin was designed. The behavior of HL-60 cells rolling under flow was analyzed using a high- resolution visual tracking system. This behavior was then correlated to a number of established predictive models. The combination of computational modeling and widely available fabrication techniques described herein represents a crucial step toward the successful development of continuous, label-free methods of cell separation based on rolling adhesion.

Tissue Engineering

Bone regeneration in a calvarial critical size defect using polymer/mineral composite scaffolds.

Ophir Ortiz, Racquel Z. LeGeros, Joachim Kohn
Rutgers University, New Jersey Center for Biomaterials

Introduction: The objectives of this study were to prepare composites of tyrosine-derived polycarbonate with different calcium phosphates (TyrPC/CaP) and (1) correlate their physico-chemical properties, including in vitro bioactivity (manifested by the formation of apatitic layer on the composite surface) to in vivo bone regeneration in a rabbit calvarial critical size defect (CSD) model, and (2) investigate changes in CaP properties due to incorporating these into a scaffold architecture. We used dicalcium phosphate dihydrate (DCPD), CaHPO4.2H2O; octacalcium phosphate (OCP), Ca8H2(PO4)6.5H2O; beta-tricalcium phosphate (ß-TCP), Ca3(PO4)2; and synthetic bone mineral (SBM), (Ca,Mg)10(PO4,CO3)6(OH,F)2.

Results and Discussion: Results showed the following: (1) Porous (~85%), bimodal (200-400µm macropores, and <20µm micropores) interconnecting architecture in all composites; (2) dissolution rates of the composites decreased in the order: TyrPC/SBM > TyrPC/DCPD > TyrPC/ ß-TCP > TyrPC/OCP; (3) all composites formed an apatite layer after immersion in FBS indicating in vitro bioactivity; (4) TyrPC/OCP composite scaffolds outperformed the three other scaffold types. The amount of bone regenerated by the various types of composite scaffolds was found to vary with respect to the type of CaP used (Fig. 2). In the case of the scaffold with OCP, the average trabecular bone volume regenerated was 55mm3, which is higher than the volume produced by two commercially available scaffolds based on ß-TCP (unpublished work). Additionally, it regenerated roughly half of the volume of calvarial trabecular tissue (~100mm3) that was removed to produce the 15mm defect (data not shown).

Conclusions: Results indicate that the TyrPC/CaP composites tested have potential as scaffolds for tissue engineering in bone regeneration. All composites evaluated in this study showed in vitro bioactivity. The TyrPC/OCP scaffolds showed the highest amount of bone regeneration after the six week time point. This may be attributed to the dissolution rate of this particular composite, which provided the appropriate calcium and phosphorus release rate for the cascade of biological processes to occur.

More Efficient Generation of Human Induced Pluripotent Stem Cells from Fetal Hepatocytes than Adult Hepatocytes in Feeder-Free Conditions

Marc C. Hansel, Roberto Gramignoli, William Blake, Julio Davila, Kristen Skvorak, Kenneth Dorko, Veysel Tahan, Edgar Tafaleng, Jorge Guzman-Lepe, Alejandro Soto-Gutierrez, Ira J. Fox
Unviersity of Pittsburgh

Introduction: Hepatocyte transplantation is being considered as an alternative to orthotopic liver transplantation for certain liver diseases. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) may be a useful source of hepatocytes for basic research and transplantation if efficient and effective differentiation protocols were developed and problems with tumorigenicity could be overcome. Recent evidence suggests that cell type of origin may affect hiPSC differentiation potential. Under this hypothesis hiPSCs, generated from hepatocytes, may differentiate back to hepatocytes more efficiently and produce cells that more closely resemble primary hepatocytes than hiPSCs generated from different cell types.

Methods: Human hepatocytes were isolated from normal and diseased liver and exposed to lentiviral vectors carrying up to 6 reprogramming genes (OCT4, SOX2, KLF4, c-MYC, NANOG, and LIN28) at a targeted MOI of 10. Reprogrammed colonies were picked based on hESC-like morphology.

Results: Forty (40) hiPSC lines were generated from primary human hepatocytes under entirely feeder-free conditions. Of these, 37 lines were generated from fetal hepatocytes, 2 additional lines from normal hepatocytes and 1 line from a patient with Crigler-Najjar Syndrome, Type-1. All lines examined expressed markers of pluripotency, including confirmed expression of OCT4, SOX2, NANOG, GDF3, hTERT, SSEA3, SSEA4, TRA1-60 and TRA1-81 at levels similar to those found on hESCs. All lines examined formed teratomas when transplanted. Interestingly, fetal hepatocytes were reprogrammed at a frequency approximately 50-fold more efficiently than adult hepatocytes. Differentiation experiments are currently underway.

Conclusions: These studies are the first to report that hiPSCs can be generated in entirely feeder-free conditions. Moreover, it is the first report of generating hiPSCs from adult and fetal hepatocytes from normal individuals and those affected with genetic diseases. Reprogramming of fetal hepatocytes is significantly more efficient than adult hepatocytes. Furthermore, fetal hepatocytes were demonstrated to be reprogrammed using only OCT4, SOX2, and NANOG. Lines of normal and genetically defective hiPSCs, and the hepatocytes derived from them, should be useful for basic research or transplantation.

Gd(III)-DNA Gold Nanoconjugates for Cell Tracking During Neural Stem Cell Implantation Therapy

Matthew Rotz, Thomas J. Meade, Francesca Nicholls, Michel Modo
Northwestern University

Stroke is currently the greatest source of adult disability in the United States, for which there is no truly effective therapy. Recent animal research has suggested that an innate population of neural stem cells (NSCs) present in mammalian brains respond to brain trauma resultant from stroke. A better understanding of this NSC behavior has the potential to be therapeutically relevant and to substantially improve the outlook of those affected by stroke. Modo and coworkers have recently shown that in rat brains, neural stem cells injected adjacent to the site of stroke translocate to the site of trauma and differentiate into the necessary cell types to help regenerate neural tissue. Currently, there is no single, effective means to visualize the movement of these cells in vivo, and thus the ultimate result of any NSC implantation procedure must be established post-mortem by histological analysis. Based on this need for an applicable in vivo imaging technique, we are developing Gd(III)-DNA gold nanoconjugates (Gd(III)-DNA AuNPs)for magnetic resonance imaging (MRI). In previous work, our lab has shown these particles to be highly effective at labeling other cell types for ex vivo labeling and cell pellet imaging by MRI. Therefore, Gd(III)-DNA AuNPs will allow the use of MRI and T1 contrast enhancement for following NSCs in vivo. We have undertaken the synthesis of a next generation Gd(III)-DNA AuNP for higher performance contrast enhancement and particle payload to investigate the viability of the nanoconjugate platform for this role. A new, click-ready gadolinium chelate has been synthesized that exhibits and optimal water exchange rate and improved r1 relaxivity per metal ion. Improving upon our previously established AuNP conjugation technique, Gd(III) payload has been improved through optimization of loading conditions and quantified by inductively couple plasma mass spectrometry (ICP-MS). Particles have further been studied for shelf life stability and relaxivity at physiological pH and temperature in cell media. With performance and stability established, particles will be incubated with NSCs to quantify cell uptake and viability. The results from these studies will drive the use of these particles in vivo for NSC implantation therapy in stroke induced rats.

Myofibroblastic response of AVICs on embryonic leaflet stiffness substrates

Young Wook Chun, Joey V. Barnett, W. David Merryman
Vanderbilt University

Stiffness has a significant effect on the function of differentiated cells as well as the direction of stem cell differentiation. Recently, it has been reported that substrate stiffness affects the function of aortic valvular interstitial cells (AVICs). Specifically, elevated stiffness leads to an increase in smooth muscle alpha actin (SMA) expression, a marker for myofibroblast differentiation. Healthy aortic valves have AVICs that express little SMA, but aortic valve disease has been shown to be characterized by an increase in myofibroblastic AVICs. Many studies in the heart valve field have been performed using AVICs that have been cultured on rigid tissue culture polystyrene (TCPS), and subsequently, it was observed that SMA expression increases with culture. Although AVICs in low passages exhibit characteristics similar to that of AVICs in situ, more accurate characterization of AVICs is needed in order to better understand their behavior. Here, we investigated the characteristics of AVICs isolated from porcine aortic valves and cultured only on hydrogels ranging in stiffness from 0.5 to 25kPa, which represent embryonic leaflet stiffness to fibrosa layer stiffness in adult leaflet, for 7, 10, and 14 days. Proliferation, cell motility, SMA expression, and response to TGF-beta1 were determined using biochemical analyses. The proliferation rate of AVICs exhibited stiffness-dependency, where the proliferation rate of AVICs on embryonic stiffness was significantly lower compared to those on fibrosa layer stiffness. Additionally, AVICs grown on the low stiffness substrates were significantly less motile than AVICs cultured on stiffer substrates. SMA expression also appeared to be stiffness-dependent with stiffer substrates leading to more myofibroblastic activation. Finally, there was no significant change of SMA expression on AVICs between adult and embryonic leaflet stiffness in the presence or absence of TGF-beta1. This indicates that AVICs on embryonic substrates, as well as adult leaflet-like stiffness minimize the expression of SMA even in the presence of TGF-beta1, although AVICs on TCPS can be affected by TGF-beta1. These data suggest that AVICs that have been cultured on TCPS may not accurately reflect the characteristics of AVICs in situ.

Systems analysis of intertissue signaling dynamics in tooth organogenesis

Daniel J. O'Connell, Joshua W. K. Ho, Peter J. Park, Richard L. Maas
Harvard Medical School

Knowledge of the mechanism that underlies the spatiotemporal regulation of developmental signaling pathways across multiple cell types is a prerequisite for designing rational approaches to regenerate any multi-tissue organ. Development of many organs depends on the sequential and reciprocal exchange of various signaling molecules between juxtaposed epithelial and mesenchymal tissue, but the detailed mechanism controlling these epithelial-mesenchymal (E-M) interactions remains unknown. We used the developing mouse molar tooth as a highly tractable model to decipher the gene regulatory network (GRN) that underlies the complex E-M signaling dynamics during organogenesis. Through the extensive profiling of over 100 microdissected embryonic mouse dental E-M tissues, mutant tissues and signaling molecule treated tissues, our analysis reveals two surprising new insights: (1) Despite the reciprocal exchange of signaling molecule expression, the overall temporal genome-wide expression change in E-M tissues is highly concordant, and (2) among key signaling pathways, the Wnt and Bmp pathways are the primary driver of odontogenesis. We developed a statistical approach to integrate our expression datasets with over 1,000 pieces of perturbation evidence from the literature to generate an inter-tissue GRN for early odontogenesis. Within this GRN, we identified a novel feedback circuit that connects the Wnt and Bmp pathways across the E-M tissue compartments through the action of the Wnt and Bmp4 ligands. Moreover, our inter-tissue Wnt/Bmp circuit was validated with two sets of in vivo mouse genetic crosses designed to “short-circuit” or to “break” the feedback circuit. Computer simulation demonstrates that the circuit structure alone can account for the observed signaling molecule expression dynamics in wild-type and mutant mice. This work illustrates how complex signaling dynamics like the E-M interactions in organogenesis represent an intrinsic property of the underlying GRN structure. Since E-M interactions in early odontogenesis resemble those in other organs, our findings will advance efforts aimed at multi-tissue organ regeneration. We developed a web-based resource called ToothCODE (http://compbio.med.harvard.edu/ToothCODE/) to facilitate public access to the genomic data generated in this work.

O'Connell DJ*, Ho JWK*, Mammoto T, Turbe-Doan A, O'Connell JT, Haseley PS, Koo S, Kamiya N, Ingber DE, Park PJ, Maas RL (2012) A Wnt-Bmp feedback circuit controls intertissue signaling dynamics in tooth organogenesis. Science Signaling, 5, ra4 [Featured on the cover of this issue of the journal] [*Co-first author]

Delivery of Platelet-Derived Growth Factor from Bone-Mimetic Electrospun Matrices as a Chemotactic F

Matthew C. Phipps, Yuanyuan Xu, MD, Susan L. Bellis, PhD
University of Alabama at Birmingham

Purpose: Although bone has a dramatic capacity for regeneration, certain injuries and pathologies present bone defects that require surgical intervention to heal. These procedures utilize autografting, the painful process of harvesting a patients bone tissue, and implanting it into the defect. Researchers have turned towards the use of biomaterials to replace the need for autograft. Utilizing a process known as electrospinning, our lab has developed a bone-like nanofibrous matrix consisting of the mechanically stable polymer polycaprolactone (PCL), and the natural bone matrix molecules collagen I (col) and nano-hydroxyapaptite (HA). Previously we have shown that these matrices support greater mesenchymal stem cell (MSC) adhesion, proliferation and in vivo bone formation compared to electrospun PCL alone. The recruitment and support of MSCs, the multipotent bone progenitor cells within bone marrow, is vital to the bone healing process. To stimulate MSC recruitment into the site of the skeletal defect, this study focuses on the sustained local delivery of Platelet Derived Growth Factor-BB (PDGF-BB), a potent chemokine for MSCs, from PCL/col/HA bone-mimetic matrices (BMM).

Methods: Scaffolds were produced by electrospinning solutions made in hexafluoroisopropanol at 7.5% solution weight: 100% PCL and 50% PCL + 30% col + 20% HA. Chemotaxis assays with human MSCs were performed in Boyden chamber units in response to soluble growth factors. MSC migration was also measured visually in a modified scratch assay. Scaffolds were soaked in PDGF-BB and protein adsorption and release was measured via ELISA.

Results: An initial survey of different growth factors in Boyden chamber chemotaxis assays revealed that PDGF-BB had the greatest effect on MSC migration. Additionally, BMMs adsorbed significantly more, and subsequently released more PDGF than PCL scaffolds for 8 weeks in vitro. Released PDGF maintained its bioactivity, stimulating MSC migration in Boyden chamber assays as well as in a custom migration assay with PDGF/BMMs placed 1.5cm from the edge of a distinct cell front created in a monolayer of MSCs.

Conclusions: These collective results combined with our prior work suggest that the inclusion of col and HA in bone-mimetic scaffolds not only facilitates greater MSC adhesion and proliferation, but also increases the adsorption and subsequent release of growth factors for sustained local delivery, much like natural bone ECM. Through the delivery of PDGF-BB from our BMMs, we ultimately present a biomaterial capable of stimulating chemotaxis as well as supporting the adhesion and survival of MSCs.

Targeted Insertion of a Selectable Lineage Tracing Reporter in Human Pluripotent Stem Cells

Jay Gantz, Nathan Palpant, Robert Welikson, Stephen Hauschka, Charles Murry, Michael Laflamme
University of Washington

PURPOSE: To create a novel undifferentiated hESC line that contains a selectable "stoplight" fluorescence reporter suitable for Cre-lox based fate mapping studies.

METHODS: Homologous recombination and even stable transfection in human pluripotent cells are extremely difficult, while integrating viral vectors result in an unknown number of inserted copies and are susceptible to transgene silencing and positional effects. To address these issues, zinc-finger nuclease (ZFN) technology was used to stably insert a single copy of a fluorescent "stoplight" Cre-lox reporter construct into a known locus in human embryonic stem cells (hESCs). For this, a previously described floxed reporter, the mTmG transgene (Muzumdar et al Genesis 45: 593-605, 2007), was modified by the addition of a cassette for the antibiotic selection of Cre-recombined cells and flanking homology arms suitable for targeting it to the human AAVS1 locus. Gene targeting to the AAVS1 locus minimizes silencing, positional effects and off-target interference with cellular function in hESCs (Hockemeyer et al. Nat Biotechnol 27: 851-857, 2009). In cells successfully targeted with this construct (hereafter referred to as "mTmG-2a-Puro"), Cre recombinase expression permanently induces a switch from constitutive expression of the red fluorescent protein tdTomato to constitutive expression of eGFP and puromycin antibiotic resistance.

RESULTS: The mTmG-2a-Puro reporter was successfully targeted to the AAVS1 locus in undifferentiated RUES2 hESCs, with site-specific integration verified by Southern blot. To assess the kinetics of the fluorescence transition after Cre expression, we transduced the resultant mTmG-2a-Puro hESCs with a lentivirus expressing Cre under the constitutive EF1a promoter and tracked the percentage of red and/or green fluorescent hESCs by daily flow cytometry. eGFP+ cells were first detected at 24 hours and became a distinct population from tdTomato+ cells over several days. The eGFP+ cells were isolated by treating the mixed population with puromycin, resulting in a nearly 100% eGFP+ tdTomato- population. To test the function of our system in differentiated hESC progeny, we transduced differentiated cultures (containing ~60% cardiomyocytes) with a lentiviral vector that encodes Cre recombinase under the control of the striated muscle specific MCK-CK7 promoter. eGFP+ a-actinin+ cardiomyocytes were then selected with puromycin to greater than 98% purity.

CONCLUSION: We have successfully created a novel undifferentiated hESC line that contains a convenient "stoplight" fluorescence reporter suitable for Cre-lox based fate mapping studies. This system should be useful in the purification of any cell population that has been indelibly marked by Cre recombinase expression.

A miRNA expression signature associated with Wnt/BMP4 GRN during molar morphogenesis

Ivan T. Rebustini, Daniel J. O'Connell, Joshua W. K. Ho, Xiu-Ping Wang, Richard L. Maas

Brigham and Women's Hospital; Harvard Medical School

Non-coding micro-RNAs (miRNAs) target the expression of multiple genes, and provide an important regulatory level in a variety of developmental processes. The embryonic mouse molar is a premier model system to investigate epithelial and mesenchymal interactions during organ morphogenesis. Signaling pathway manipulations have demonstrable effects on molar morphogenesis, including constitutive Wnt activation that results in supernumerary tooth formation, and disruption of BMP4 signaling, which results in oligodontia. A Wnt/BMP4 Gene Regulatory Network (GRN) has been proposed to integrate these signaling pathways during early molar morphogenesis. However, this GRN does not include miRNAs. The purpose of this study is to elucidate the identity and the function of miRNAs in regulating Wnt and BMP GRN during early odontogenesis. Methods: We adopted a comparative approach using low-density qPCR arrays and multiple embryonic organs, to detect a miRNA expression signature unique to early molar morphogenesis. We used two mouse models of Wnt activation, the adenomatous polyposis coli loss-of-function (Apccko/cko) and the beta-Catenin activation (Ctnnb Exon3/flox), to investigate the changes in miRNA expression associated with supernumerary tooth formation. Results: we found that miR-590-5p is enriched in the embryonic molar when compared to other embryonic organs, and that miR-590-5p is downregulated in the mouse models of constitutive Wnt activation. We identified several predicted miR-590-5p target genes, including Msx1 and Smad7, which are components of Wnt and BMP signaling pathways, and Timp3 and Chd7, which regulate extracellular matrix and chromatin remodeling, respectively. Conclusions: our results suggest that a unique miRNA expression signature regulates Wnt and BMP signaling and epigenetic state during early molar morphogenesis.

Stem Cell Therapy: Not All Approaches Are Created Equal

Mai T. Lam, Yongquan Gong, Joseph C. Wu, Michael T. Longaker
Stanford University

Introduction: The general method for applying stem cells in a therapy is through injection into the wound site. Though this method has been used extensively, it is ineffective because the stem cells tend to dissipate from the wound area or die. A more effective method for delivering stem cells would increase their translational potential. Our approach is to transplant stem cells using a biomaterial to retain them locally and to support their survival. We investigated the efficacy of a natural biomaterial derived from porcine small intestine submucosa (SIS) as a stem cell delivery vehicle and support structure.

Methods: We tested our stem cell delivery system in skin wounds and heart injury models to compare treatment efficacy in distinctly different wounds. In the skin, a wound healing model was created in mice and wounds treated with adipose-derived stem cells (ASCs), with experimental groups consisting of stem cells alone, stem cells delivered with the patch, and the patch alone. In the heart, a myocardial infarct model was created in mice and the infarcted region treated with cardiosphere-derived cells (CDCs), with experimental groups consisting of patch alone and patch with stem cells. Imaging modalities were used to analyze results of the stem cell treatment, including confocal, bioluminescence imaging, and echocardiography. Histology revealed evidence of scar tissue and tissue structure post-healing.

Results: Healing results varied greatly depending on the type of tissue wounded. In skin wounds, ASC survival and proliferation was markedly increased with addition of the patch. Patch only treatment stented the wound open, resulting in slowest healing rates and highest amounts of scar tissue. Treatment with the cell-seeded patch with removal of the patch 6 days after application resulted in the most improved skin healing as evidenced by reduced amounts of scarring. In contrast, in the heart, patch treatment alone resulted in the highest recovery of cardiac function compared to patches with stem cells, as shown by cardiac output results. This is due to the transplanted stem cells prematurely degrading the patch on the heart, resulting in a thinner heart wall, and thus hindering pumping capabilities. These results reveal that stem cell treatments need to be customized for each specific wound type.

Conclusions: In some cases, a stem cell treatment approach is optimal for recovering tissue function, whereas in others a purely biomaterials approach is better. These differences are attributed to the structure-function relationship of the specific organ.

Analysis of Cellular Rigidity Sensing Using Composite Materials

Stephanie Wong, Wei-hui Guo, Yu-li Wang
Carnegie Mellon University

Purpose: Regenerative medicine can benefit from a thorough understanding of cellular mechanisms as the foundation to efficiently and effectively develop clinical treatments. Rigidity sensing of adherent cells is thought to be important in wound healing, stem cell differentiation, and other physiological processes. However, the biophysical machinery allowing cells to migrate toward rigid substrate, termed durotaxis, is poorly understood. Detection of durotaxis by time-lapse recording of cell migration is very time consuming and inefficient, which represents a serious obstacle in understanding its mechanism or screening for conditions that impair the process.

Methods: To analyze the mechanism of durotaxis, we have developed a micropatterned composite material that consists of rigid adhesive islands of photoresist grafted on top of soft polyacrylamide hydrogels. A large island provides a stiff area for cell spreading, while adjacent small islands create the transition to an easily deformable soft area. We predict that normal cells will stay on the large island, while cells defective in durotaxis would spread across the soft hydrogel onto the adjacent islands. Control material without a steep transition in rigidity was created by grafting similar photoresist islands on stiff hydrogels.

Results: NIH 3T3 cells cultured overnight on the composite substrate were largely confined to the stiff area of large islands, with minimal spreading across soft hydrogels onto the adjacent small islands. Cells on control substrate were able to spread across stiff hydrogels and occupy most or all of the adjacent small islands. FAK knock out cells (FAK -/-), known to be defective in rigidity sensing, were able to spread across the whole pattern and take most or all of the small islands regardless of the stiffness of the hydrogels. Upon re-expression of FAK, the cells regained rigidity sensitivity and were no longer able to spread across soft gels. Expression of the H-ras oncogene in NIH 3T3 fibroblasts also impaired rigidity sensing and caused a similar defect as seen with FAK -/- cells.

Conclusions: We have developed a new model system to easily screen both motile and non-motile cells for their responses to rigidity gradient. We found that FAK is required for durotaxis. In addition, oncogene expression can impair rigidity sensing, which may play a role in metastatic invasion. Our approach may be applied to explore the mechanism of cellular mechanosensing, which may in turn lead to improved strategies for regenerative medicine or cancer treatment.

Engineered macrophages for the application of a healing cardiovascular tissue engineering scaffold

Karen V Eaton, Mei-Ting Wu, Hsueh YL Yang, Marta Scatena, Cecilia M Giachelli
University of Washington

Purpose: It has been estimated that cardiovascular disease and subsequent heart attacks have claimed about 17.1 million lives worldwide in 2010 alone and as such, is an important target for new therapeutic developments. A major problem following a myocardial infarction is the resultant non-regenerative dead cardiac tissue caused by coronary artery blockage. Regenerative technologies and treatments for this condition are necessary to regrow heart tissue in these necrotic regions, in order to restore or improve heart function. Implantation of functional cardiac cells has been a proposed therapy to damaged heart tissue after myocardial infarction; however this method only replaces existing dead cells and lacks control of the healing process. We propose an additive component to this therapy, in which engineered macrophages are added to the cardiac cell scaffolds in order to facilitate cardiac tissue regeneration by controlling both the inflammatory and host response.

Methods: Macrophages can be polarized in vitro to a M1 phenotype via a lipopolysaccharides (LPS)-Toll-like Receptor 4 (TLR4) interaction, and to a M2 phenotype via an Interleukin 4 (IL-4)-Interleukin receptor (IL-4R) interaction. In order for these ligand-receptor interactions to activate their respective pathways, it is necessary for both of these membrane-spanning receptors to dimerize. Physiologically, receptor dimerization is achieved by ligand binding; however, protein engineering utilizing the chemically induced dimerization (CID) system has been employed to activate these pathways in the absence of their specific ligands. This CID system works by fusing the intracellular receptor domain to an F36V domain, which binds to a diffusible synthetic ligand (AP20187 or AP21967) that is a chemical inducer of dimerization. Creation of these engineered macrophages will potentially allow for the selective and temporal polarization of M1 and M2 macrophages for the development of a controlled tissue engineering construct for cardiovascular repair that can both rebuild cardiac tissue and regulate healing.

Results/Conclusions: To test for the polarization of engineered macrophages into a M1 or M2 macrophage phenotype through the induced activation of the TLR4 or IL4R pathway, respectively, key cell markers are being measured. Induced M1 macrophages are being tested for iNOS and IL-12 markers via cell cytometry analysis. Likewise, induced M2 macrophages are being tested for mannose receptor via cell cytometry analysis and Arginase 1 activity via an arginase assay.

Characterizing Forces Exerted by Mesenchymal Stem Cell Aggregates During Tissue Regeneration

Rachel Simmons, Michelle Dawson
Georgia Institute of Technology

Purpose: The delivery of stem cells and soluble factors via engineered hydrogels is becoming a popular method for tissue regeneration. In particular, mesenchymal stem cells (MSCs) in conjunction with TGF-beta1 have been used to promote chondrogenesis in scaffolds (Li, 2009). In load-bearing areas, the hydrogel must accommodate stress imposed by the body. Acellular hydrogels are often used in these applications; however, in certain situations it may be advantageous to use the hydrogel as a delivery system for engineered cells. Incorporated cells may alter the mechanical properties of the hydrogel by secreting enzymes that degrade the material or by exerting force that enhances the mechanical rigidity of the network. In previous studies, we found that soluble factors, such as TGF-beta1 and PDGF, increase the mechanical rigidity of individual MSCs; however, little is known about their effects on force generation by MSC aggregates in tissue engineering scaffolds or hydrogels. To this end, we will study the growth profile of MSC spheroids in a simple hydrogel, agarose, as well as quantify the strain exerted by the spheroids under the influence of various growth factors, including TGF-beta1 and PDGF. Forces exerted by MSC spheroids will be quantified using a bead displacement model (Cheng, 2009). This information will be used to guide the rational design of cell-based scaffolds for soft tissue injuries.

Methods: GFP-expressing C57BL/6 MSCs were cultured with IMDM (10% FBS, 10% HS) in 2% (w/v) agarose (Type VII low gel, Sigma Aldrich) cast from micromolds (Microtissues) for 24 hours to form MSC spheroids. The viability of cells in the spheroids was determined by propidium iodide staining. The spheroids were recovered from the micromolds by centrifugation and then mixed at a 1:1 volumetric ratio with a 1% (w/v) agarose solution, which contained 0.02% 1 micron fluorescent red particles. The resulting 0.5 % (w/v) agarose solution was cast into an 8-well chamber slide and allowed to polymerize for 10 minutes, after which time, the wells were filled with control media or media containing growth factors. Bead displacements were monitored over a 1-week period using a fluorescent microscope with 40x-oil immersion lens.

Results: Under normal growth conditions, spheroid size did not increase after 24 hours. We are in the process of studying the effects of growth factors on spheroid size and force generation.

Conclusion: MSCs can be formed into viable spheroids in the presence of agarose. Spheroid growth is limited, which may indicate differentiation.

Regulatory Network Discovery Using Temporal DNase-seq

Charles W. O'Donnell, Tatsu Hashimoto, Sophie Lewis, Richard Sherwood, Douglas A. Melton, David K. Gifford

Harvard University; Massachusetts Institute of Technology

Purpose: The ability to reprogram diverse cell types into therapeutically useful or disease-relevant states remains a core goal of regenerative medicine. Directed differentiation and transdifferentiation approaches have made great progress toward this end by identifying cell-type specific master regulator genes, however, discovering such factors can be a painstaking process plagued by issues of efficiency, heterogeneity, and competence. Our goal is to rationally design more reliable reprogramming strategies based on an understanding of the signaling and transcriptional networks activated during normal development. Specifically, we model the spatial relationship of transcription factor (TF) binding events within genomic enhancers and promoters, and how these relationships change over time in embryonic stem cell (ESC) directed differentiation. Common enhancer TF binding patterns, or grammars, are linked with gene expression and other markers to posit functional relationships. From these we hope to discover novel regulatory circuitry as well as identify existing networks amenable to manipulation.

Methods: To probe the genome-wide configuration of TF/enhancer states we rely on a hypersensitivity assay called DNase-seq: Here, nuclear cell extract from defined stages of differentiation is exposed to DNase-I, which preferentially cuts 'open' chromatin according to adjacent TF occupancy and active histone configurations. Sequencing this DNA reveals enzyme sensitivity patterns from which we infer the existence of population-wide protein binding, histone coordination, and other factors effecting DNase-I cleavage. By incorporating sequence information and biophysical parameters we are able to computationally predict which protein causes each TF binding occupancy and its context.

Results: In preliminary mouse ESC DNase-seq data, hypersensitive regions are found to co-locate with the binding sites of known ESC TFs roughly 80-90% of the time (as identified by ChIP-seq). Further, sub-classifications of binding event types is evident, and hypersensitive regions suggest bivalent chromatin. Our current algorithm is able to recover 90% of motif-associated ChIP-seq binding events given an FDR of 10%, and has identified novel, lineage-specific changes in TF binding patterns over time.

Conclusions: Statistical analysis of DNase-seq data has been shown to be a unique and efficient tool for probing chromatin state and potential TF binding. The integration of directed differentiation time series data into a unified model can help further identify differences in lineage-specific enhancers and promoters.

Characterization of the incisor stem cell niche using gene co-expression network analysis

Kerstin Seidel, Michael Oldham, Richard Maas, Ophir Klein
University of Californa, San Francisco

The continuously growing mouse incisor provides a unique system for studying the biology of adult stem cells for a number of reasons. Its unidirectional growth, with progeny at progressively increasing stages of maturity arrayed in a linear fashion, enables dissection of stage-specific mechanisms. Since the basic processes required during generation of progeny from stem cells are likely shared with several other systems, such studies of the incisor will produce generalizable results. Furthermore, the incisor is an organ with high cell turnover that consists of epithelial and mesenchymal cell types, allowing for investigations of how stem cell pools in both tissue compartments are integrated in order to ensure generation of distinct cell lineages in a coordinated fashion. To date, in-depth analysis at the cellular level has been hindered by our limited understanding of the cellular diversity present in the incisor and by the absence of markers that allow a clear discrimination between the distinct cell types. Therefore, we set out to identify novel, cell type-specific markers by analyzing the gene co-expression network organization in the proximal incisor. In this study, expression profiles were generated from 96 individual tissue samples micro-dissected from the proximal incisor region of 96 wild-type adult mice. These expression data are being used to perform gene co-expression network analysis to identify modules of co-expressed genes. Previous work has shown that gene co-expression modules identified in heterogeneous tissues frequently relate to distinct biological processes and are often driven by discrete cell types; therefore, we will investigate whether the identified modules are enriched for the few known markers of specific cell types in the tooth by cross-referencing published gene expression data. Cell type specificity will be validated histologically by visualizing gene expression patterns of the highest ranked factors contributing to each module. Analysis of data from a pilot study performed with a smaller dataset has yielded promising results, such as the identification of a module that appears to be specific to the population of transient-amplifying cells. The wealth of new information obtained by this study will greatly enhance our understanding of the cellular composition of the incisor system and facilitate our studies of adult stem cells by rendering effects of experimental perturbations more interpretable.

Towards quantification of secreted matrix metalloproteinases during branching morphogenesis

Somin Lee, Paul Alivisatos, Mina Bissell
Lawrence Berkeley National Laboratory

PURPOSE: Branching morphogenesis is a dynamic process used by the organism to form a variety of organs, such as kidneys, salivary glands, and the mammary glands. Matrix metalloproteinases (MMPs) have been identified previously as key mediators of branching morphogenesis in the mammary gland presumably by degrading the extracellular matrix as cells invade into the fat pad. However, it is still unclear how these proteinases signal to provide patterning information to the branching tissue over time. To answer this question, methods for long term, real time, and quantitative measurement of secreted proteinases in the local microenvironment are needed but are currently lacking. We propose to employ plasmon rulers - consisting of peptide-linked noble metal nanoparticles - which are capable of long term, real time, and quantitative measurements of secreted proteinases, for the purpose of understanding the branching process and engineering branched structures.

METHODS: Mouse mammary epithelial cells were maintained in 1:1 DMEM/F12, 2% FBS, 5 ug/mL insulin, and 50 ug/mL gentamycin (Sigma). 3-D cultures were prepared by growing cells to confluence as monolayers, followed by trypsinization, and embedment into collagen (3 mg/mL) matrix with 1:1 DMEM/F12 media supplemented with ITS and penicillin/streptomycin. 9 nM growth factor TGFa was introduced to induce branching morphogenesis. 20 mM phenanthroline (Sigma) was used to for general inhibition of MMPs and 50 uM MMP3-specific peptide-based inhibitor (Calbiochem) was used for specific inhibition of MMP3. For analysis of MMP3 protein, samples were lysed using modified RIPA buffer, mixed with sample buffer, heated at 95C for 5 minutes, resolved by SDS/PAGE,and transferred to nitrocellulose. Primary antibodies to MMP3 (R&D systems) were detected with the Pierce SuperSignal detection kit and the FluorChem 8900 analysis system (Alpha Innotech). For analysis of MMP3 activity, degradation of a fluorogenic casein peptide (Invitrogen) was measured over time using a fluorometer (Tecan).

RESULTS AND CONCLUSIONS: We have identified MMP3 as the MMP candidate for long term and real time quantification during branching morphogenesis. We have detected MMP3 protein using western analysis during TGFa–induced branching morphogenesis in 3-D culture. We have quantified MMP3 activity in 3-D culture using fluorogenic casein peptides. Based on protein and activity analyses, we are currently designing recognition sequences to MMP3. Plasmon rulers consisting of peptide-linked noble metal nanoparticles will be developed for use as bioimaging probes to identify activity profiles of secreted MMP3 and investigate how MMP3 may provide patterning information to a branching tissue over time.

Investigating the role of morphogens in early tooth formation through in vitro 3D gradient system

Shilpa Sant, Chia-Cheng Li, Abinaya Ravisankar, Richard L Maas, Ali Khademhosseini

Brigham and Womens Hospital

Introduction: Mammalian tooth formation (odontogenesis) is induced and regulated by a sequence of reciprocal epithelial-mesenchymal interactions. Critical signaling events occur at the initiation and bud stages, during which inductive morphogens from the epithelium such as Bone Morphogenetic Protein 4 (Bmp4) diffuse and form gradients to activate odontogenesis within the underlying mesenchyme. However, the interactions among various morphogens and their concentrations have not been addressed so far.

Naturally derived hydrogels mimic natural tissues and provide an optimal 3D microenvironment for cell attachment, growth and function. Various techniques such as microfluidics and microscale engineering can be applied to synthesize biomimetic hydrogels with tailored characteristics. Using simple capillary flow in a fluid stripe, we have established an in vitro platform, which involves the generation of Bmp4 gradients within cell encapsulated gelatin methacrylate (GelMA) hydrogels, and used this approach to study the early odontogenesis.

Materials and Methods: In this study, we first established an in vitro gradient system using GelMA hydrogel and characterized gradient formation using FITC-dextran and FITC-BSA as model molecules. We also investigated the effects of pre-wet and droplet volumes on gradient formation and on the biocompatibility of NIH 3T3 cells encapsulated in gradient hydrogels. We further encapsulated mandibular mesenchymal cells harvested from embryonic day 10.5-11.0 (E10.5-E11.0) mouse embryos within the hydrogel and examined their biocompatibility by Alamar Blue assay, and their response to Bmp4 morphogen stimulation by qRT-PCR.

Results and Discussion: According to our results, a higher droplet volume (Vd)/pre-wet volume (Vw) ratio resulted in gradients over longer lengths. Linear gradients were generated using Vd/Vw ratios of 1/6 and 1/4. Uniform encapsulation of cells within gels did not have any effect on the length or on the slope of the gradients. Gradients generated using higher concentrations of FITC-BSA resulted in similar gradient slopes and lengths. Mandibular mesenchymal cells (E10.5-E11.0) encapsulated in 5% GelMA maintained their viability in the gel. Our preliminary data indicates that encapsulated cells can respond to Bmp4 and upregulate the gene expression of Msx1.

Conclusions: In this project, we integrate tissue engineering techniques and concepts from developmental biology to establish and optimize a 3D gradient hydrogel system, which can be further applied to investigate the interactions among multiple morphogens such as Bmp4, Fgf8, and Wnt during odontogenesis.

Platelet Derived Growth Factor Receptor Alpha (PDGFR?): Dispensable or important for liver regeneration?

Prince Awuah, Kari Nejak-Bowen, Amalea Misse, Sucha Singh, SP Monga
Unviersity of Pittsburgh

Liver regeneration (LR) after partial hepatectomy (PHx) is a complex process that requires intricate and precisely timed involvement of many growth factors and cytokines secreted by multiple cell types. While platelet derived growth factor (PDGF) has been shown to be secreted by hepatocytes during LR, we report enhanced PDGFR expression and activation in regenerating mouse liver especially at 24 hours (24h). Hepatocyte-specific PDGFRA conditional knockout mice (KO) lack an overt phenotype and show serum biochemistry. When subjected to PH, KO's demonstrate a significant decrease in PDGFR? expression when compared to wild-type littermates (WT). Loss of PDGFR? in hepatocytes did not affect survival after LR. Intriguingly, while hepatocyte proliferation is comparable in KO and WT at 48h, KO showed a significantly higher number of hepatocytes in S-phase at 72h. We identified a dramatic increase in total and activated EGFR and MET proteins in KO at 24h during LR. Functional EGFR up-regulation was observed in human hepatoma cells following blockade of PDGFR? signaling. While PDGF-CC, a selective ligand of PDGFR?, promoted DNA synthesis in mouse hepatoma cells and its blockade had a converse effect, their effects on primary mouse hepatocytes were inconsequential. Although modest, PDGFR? blockade enhanced while its activation suppressed the mitogenic effects of EGF and HGF on primary hepatocytes. Thus, we report temporal activation of PDGFR? during normal LR. We have also uncovered an escape mechanism after PDGFR? suppression, which may have significance in targeted therapies in cancer. Funded by CATER fellowship (NIH T32 EB001026-05) to PA; 1R01DK62277 and 1R01CA124414 to SPM

Advanced Biomaterials

Electrospun Janus Meshes

Kristie M. Charoen, Mark W. Grinstaff
Boston University

Purpose: The goal of this project is to create a biologically relevant graft material with Janus-type dual functionality. One side of this mesh would prevent leakage of biological fluids and the other side would allow for cell ingrowth. An electrospun mesh with two distinct sides, one hydrophobic and the other, hydrophilic, would allow for both properties. The hydrophobic side could function as a barrier to maintain separation of biological fluids whereas the hydrophilic side would act as a scaffold for future cell ingrowth.

Methods: Two polymers were synthesized from a Poly(glycerol-co-e-caprolactone) (PGC) starting material. One polymer was functionalized with a hydroxyl groups (PGC-OH) to give it hydrophilic properties whereas the second polymer was functionalized with an octadecyl alkyl chain (PGC-C18) to instill it with hydrophobicity. Polycaprolactone was electropsun with 10% doping of either PGC-based polymer. Electrospinning parameters such as polymer concentration, collection distance, voltage and ejection rate were tuned to obtain bead-free fibers. Resulting meshes were characterized via scanning electron microscopy (SEM) and contact angle.

Results: SEM was used to determine fiber morphology and size. The hydrophilic polymer yielded meshes with a homogeneous population of micron-sized fibers whereas the hydrophobic polymer produced meshes with a bimodal distribution of micron and nanometer sized fibers. Both meshes demonstrated bumpy fibers. Contact angles for PGC-OH and PGC-C18 doped meshes were 36.1±3.3 and 134.8±1.9 degrees, respectively. Contact angles were taken at 30 seconds after initial mesh-droplet contact, otherwise PGC-OH demonstrated complete wetting.

Conclusions: Two polymers were synthesized and successfully electrospun into bead-free fibrous meshes. The two components have the desired hydrophobic and hydrophilic characteristics necessary for a double-sided Janus mesh. Future work would combine the two meshes to look at side specific cell interactions and protein binding.

Adeno-Associated Virus Nanoparticles as Scaffolds For Gold Nucleation

Chris Dempsey, Rebekah Drezek, Dave Evans, Junghae Suh
Rice University

Purpose: Being able to control the nucleation of metals at the nanoscale could have far reaching implications in material synthesis. Utilizing proteins as scaffolds upon which these syntheses can occur is a powerful approach for finely controlling the orientation, shape, and size of the final material. By using one such protein scaffold, Adeno-Associated Virus (AAV), we have created virus-gold nanoparticles (AAV-Au) that were examined for various physicochemical properties. Gold nanoparticles (AuNP) have been used for their novel optical properties in biological imaging Depending on their shape and size, AuNP can have drastically different absorption and emission spectrums, which makes them good candidates for in vivo imaging.

Methods: A solution of gold hydroxide was made using gold(III) chloride trihydrate in water and adding potassium carbonate. AAV purified in DPBS was used for the AAV gold reaction. Gold and virus or a negative control of water and virus was rocked for 2 hours wrapped in foil at room temperature. A freshly prepared solution of hydroxylamine hydrochloride was then added to make a final concentration of 20 mM for 30 minutes followed by mounting on copper mesh transmission electron microscopy (TEM) grids and immediate size measurement using dynamic light scattering (DLS).

Results: 500 µL of 3E11 titer virus mixed with 10 µL of gold hydroxide followed by reduction results in a population of relatively monodispersed, round nanoparticles around 200 nm in size. The negative control reaction results in morphologically flat and jagged particles. This is a promising indication that the virus capsid is acting as a scaffold for gold hydroxide ions to attach to whereupon they can be reduced to form the spherical particles. Reaction parameters were varied in order to determine their effects on particle size and morphology. These conditions include reduction time, reaction volume, and dilution. Shell-like particles were observed from the reactions, which is indicative of gold using virus particles as scaffolds for nucleation.

Conclusions: By using AAV as a scaffold for gold nucleation, we have shown the innate properties of the virus capsid are sufficient to induce binding of the gold ions that can be reduced to form a shell. We plan to utilize a higher titer virus sample to try to obtain a more monodispersed population of particles that we will then characterize with other methods such as UV-Vis spectroscopy to determine if these particles behave differently than other AuNPs.

Microwave Plasma CVD Diamond Employing Interlayers on 440c and 316 Stainless Steel

Jared Ballinger, Shane Aaron Catledge, Vinoy Thomas
University of Alabama at Birmingham

Diamonds extreme hardness and resistance to wear make it the perfect coating material in the cutting and drilling industries. The deposition of diamond onto primarily ferrous substrates currently results in a poorly adhered film. A number of factors are responsible due to the high temperatures used in microwave plasma assisted chemical vapor deposition (MPCVD) to achieve diamond growth: carbon readily diffuses into the stainless steel substrate resulting in low nucleation density, iron diffuses outward to the surface catalyzing the growth of sp2 bonded carbon, and large residual stresses are present due to the difference in thermal expansion coefficient. 316 stainless steel disks and 440C stainless steel bearings were used in this work. Titanium nitrided (TiN) coated bearings were the first interlayer tested for effectiveness at growing diamond films. Surface boriding of bearings and disks was also conducted as a second interlayer via MPCVD. Boriding relied on a feedgas mixture of diborane and hydrogen using various temperatures. Diamond deposition studies after seeding of the interlayered stainless steel samples focused on the effects of temperature and methane concentration. Glancing angle X-ray diffraction (XRD), atomic force microscopy, scanning electron microscopy, optical microscopy, and Raman spectroscopy were implemented to characterize the borided interlayers and diamond films. Both interlayers were adequate diffusion barriers allowing for the growth of diamond. Films grown on the TiN interlayers resulted in heavy delamination upon cooling with only flakes remaining with poor adherence. This is due to no interfacial carbide present as determined by XRD and thermal expansion coefficient mismatch. However, lower temperatures and methane concentrations were found to be beneficial to film quality and adherence. Variation in temperature of the boriding stage resulted in a combination of either CrB, Fe2B, or both phases present in the interlayer. Subsequent diamond deposition on borided stainless steel produced adherent diamond films as tested by Q-tip rubbing tests. This was confirmed by the presence of Cr3C2 present at the interface via XRD. Temperature was again found to play an important role with lower temperatures resulting in more continuous films after cooling. Future work will study the interfacial bonding at the two interfaces using X-ray photoelectron spectroscopy studies and optimization of the MPCVD process parameters for continuous and adherent diamond films.

Drug Delivery

Synapse-directed delivery of immunomodulators using T-cell-conjugated nanoparticles

Matthias T. Stephan, Sirkka B. Stephan, S. Peter Bak, Jianzhu Chen, Darrell J. Irvine
Massachusetts Institute of Technology

Regulating molecular interactions in the T-cell synapse to prevent autoimmunity or, conversely, to boost anti-tumor immunity has long been a goal in immunotherapy. However, delivering therapeutically meaningful doses of immune-modulating compounds into the synapse represents a major challenge. Here, we report that covalent coupling of maleimide-functionlized nanoparticles (NPs) to free thiol groups on T-cell membrane proteins enables efficient delivery of compounds into the T-cell synapse. We demonstrate that surface-linked NPs are rapidly polarized toward the nascent immunological synapse (IS) at the T-cell/APC contact zone during antigen recognition. To translate these findings into a novel therapeutic application we tested the NP delivery of NSC-87877, a dual inhibitor of Shp1 and Shp2, key phosphatases that downregulate T-cell receptor activation in the synapse, in the context of adoptive T-cell therapy of cancer. Conjugating NSC-87877-loaded NPs to the surface of tumor-specific T cells just prior to adoptive transfer into mice with advanced prostate cancer promoted a much greater T-cell expansion at the tumor site, relative to co-infusing the same drug dose systemically, leading to enhanced survival of treated animals. Altogether, our studies support the application of T-cell-linked synthetic NPs as efficient drug delivery vehicles into the IS, as well as the broad applicability of this new paradigm for therapeutically modulating signaling events at the T-cell/APC interface.

Raman Labeled Gold Nanostars as Photodynamic Therapy (PDT) Drug Carriers for Theranostics

Andrew M. Fales, Hsiangkuo Yuan, Tuan Vo-Dinh
Duke University

Purpose: We aimed to develop a multifunctional nanocomposite that could be of use for combined therapy and diagnostics (theranostics). Gold nanostars served as both the Surface-Enhanced Raman scattering (SERS) substrate and photodynamic therapy drug carrier. The Raman dye DTTC was used to label the particles, and the photosensitizer methylene blue (MB) was loaded into a silica shell on the particles.

Methods: Gold nanostars were synthesized by a method recently developed in our laboratory. After synthesis, the particles were capped with thiol-polyethylene glycol (PEG). The PEGylated particles were mixed with DTTC overnight to label them, and then coated with silica using a modified Stöber method. MB was added to the solution during silica condensation to encapsulate the drug. Particles were characterized with TEM, UV-Vis absorption, Raman spectroscopy, and nanoparticle tracking analysis. A 785-nm laser was used to obtain SERS from the DTTC label, while a 633-nm laser was used to excite the MB for singlet oxygen generation. The singlet oxygen generation of the particles was observed using the Singlet Oxygen Sensor Green reagent from Invitrogen. To quantify the amount of encapsulated MB, the silica shell was dissolved with HF and the fluorescence intensity of MB was measured and then compared to a standard curve. In vitro efficacy was demonstrated on BT549 breast cancer cells, with 633-nm laser light fiber-coupled into an incubator and focused onto the sample with a 10x objective.

Results: This work has demonstrated the relatively strong SERRS signal from DTTC-tagged nanostars using 785-nm laser excitation. Encapsulation of MB photosensitizing drug into a silica shell around the nanostars shows increased singlet oxygen generation upon laser excitation at 633-nm compared to silica-coated nanostars without MB. It was demonstrated that the MB-loaded nanostars produce a cytotoxic effect on BT549 breast cancer cells upon laser irradiation that was not seen in the silica-coated nanostars without MB, indicating no photothermal effects.

Conclusions: This is the first report of SERS-tagged nanocomposites possessing a combined capability for SERS detection and singlet oxygen generation for PDT. These multimodal nanoprobes have potential applications in theranostics, integrating SERS imaging and PDT. Future work will further investigate the behavior of these nanoparticles in vitro and in vivo to test their efficacy as a PDT drug carrier and SERS imaging label.

Synergistic Silencing: Combinations of Lipid-like Materials for Improved siRNA Delivery

Kathryn Whitehead, Daniel Anderson, Robert Langer
Massachusetts Institute of Technology

Purpose: Despite the promise of RNA interference therapeutics, progress towards the clinic has been slowed by the difficulty of delivering short interfering RNA (siRNA) into cellular targets within the body. Nearly all siRNA delivery vehicles developed to date employ a single cationic or ionizable material.

Methods: In order to increase the material space available for development of siRNA delivery therapeutics, this study examined the possibility of using binary combinations of ionizable lipid-like materials to synergistically achieve gene silencing.

Results: Interestingly, it was found that ineffective single lipid-like materials could be formulated together in a single delivery vehicle to induce near-complete knockdown both in vitro and in vivo. Synergistic liver delivery in mice was initially observed at doses of 5 mg/kg total siRNA, with the most efficacious binary combination demonstrating an IC50 value of 1.5 mg/kg. Microscopy experiments suggested that synergistic action resulted when combining materials that respectively mediated cellular uptake and endosomal escape, two important steps in the delivery process.

Conclusions: Together, the data indicate that formulating lipid-like materials in combination can significantly improve siRNA delivery outcomes while increasing the material space available for therapeutic development.

Size-Stable Solid Lipid Nanoparticles loaded with Gd-DOTA for Magnetic Resonance Imaging

Erica Andreozzi, Angelique Louie, Marc Dhenain, Peter Wang
University of California, Davis

Purpose: Solid lipid nanoparticles (SLNs) have emerged as an efficient, non-toxic, and versatile colloidal drug carrier system that avoids some of the disadvantages of liposomes and polymeric nanoparticles and offers the potential for controlled release of cargo1. While many studies have reported SLN encapsulation of therapeutic agents, very few have reported encapsulation of diagnostic agents, especially those for imaging applications2.

Methods: Gadolinium-DOTA (Gd-DOTA), a T1-weighted contrast agent for magnetic resonance imaging (MRI), was loaded into SLNs using a water/oil/water (W/O/W) double microemulsion method3 and the resulting SLNs were characterized with dynamic light scattering (DLS) for size determination, with inductively coupled plasma mass spectrometry (ICP-MS) for Gd content, and with a Bruker Minispec relaxometer (1.4T, 37°C) for relaxivity properties (r1). T1-weighted MRI (3D gradient echo images, TR/TE/Alpha = 25/2/30°, 156 x 156 x 203 µm3, 7T) was then used to observe the positive contrast enhancement generated after intracerebroventricular (ICV) injection of Gd-loaded SLNs.

Results: DLS measurements have verified a size-controllable synthesis of a Gd-loaded SLNs with a unimodal, Gaussian distribution using a modification of the W/O/W method4, and have also confirmed the size stability over time in solution. ICP-MS analyses of dialysis experiments have confirmed that Gd-DOTA is not being released from the SLN matrix over time in the absence of brain lipases. ICP-MS has also confirmed a Gd-DOTA:lipid molar ratio = 0.09 ± 0.03 after Gd-DOTA encapsulation into SLNs. Relaxivity measurements (1.4 T, 37°C) indicate a smaller r1value (~2.5 mM-1sec-1) for the Gd-loaded SLN in comparison to free Gd-DOTA (~3.5 mM-1sec-1), which can likely be attributed to the inaccessibility of water to Gd-DOTA when encapsulated inside the SLN core. ICV injection of the Gd-loaded SLNs confirms the ability for these particles to provide positive contrast enhancement in vivo with T1-weighted MRI.

Conclusions: We showed that SLNs can be loaded with Gd-DOTA and can shorten T1 to provide T1-weighted contrast in vivo. These Gd-loaded SLNs show different relaxivity (r1) and blood clearance properties than free Gd-DOTA. Serving as stable, long-circulating, biocompatible T1 contrast agents, these Gd-loaded SLNs offer the potential to facilitate T1-weighted imaging in vivo for a wide variety of applications. Furthermore, these SLNs can be radiolabeled4 to provide high-sensitivity, high-resolution dual-modality diagnostics with positron emission tomography (PET) and magnetic resonance imaging (MRI).

5FC based gene therapy and its combination with radiation.

Gilmer Valdes, Keisuke Iwamoto, Noriyuki Kasahara, Takahashi Masamichi
University of California, Los Angeles

Introduction: Today's protocols for administration of 5FU to be used with radiation are based on Byfield et al.'s pioneering work using HeLa and HT-29 cell lines and consist mainly of continuous infussion [1, 2]. However, in previous work, we have shown that 5FU bolus injection could indeed radiosensitize U87MG VIII cells. This fact is of paramount importance when 5FU is delivered into cells through the administration of 5FC and its further conversion to 5FU after gene therapy as bolus administration is the only possible administration schedule in this case [3]. In the present work, a protocol for using gene therapy and 5FC together with radiation was outlined. This work might have great importance for current clinical trials.

Materials and Methods: Glioblastoma cell line, U87MGVIII and U87, were used to assess 5FU's and 5FC's effects on radiosensitivity. For the cell survival assays, the cells were irradiated as suspension cultures at 37oC in complete culture medium with single doses. An RS320 Irradiation System (Gulmay Medical, Bethel, CT, USA) with the following technique were used: of 300kVp, HVL 3mmCu, 10mA, 1.743Gy/min at 34.7 cm FSD.

Results: In the present work we showed that the administration of 5FU in high-dose pulses (bolus injections) does radiosensitize U87MGVIII and U87 cells through modification of alpha and beta ratios obtained from survival fraction curves. Additionally, we also showed that this effect is also obtained when 5FC is converted to 5FU inside the cells after gene therapy. Therefore, current clinical trials using gene therapy and 5FC might benefit from this fact and incorporate radiotherapy in their protocols.

Conclusions: 5 FU bolus injections could, if used appropriately, radiosensitize cells contrary to what has been suggested in the literature as it has been shown in our study. Further examination should be extended to other cell lines and to animal models.

Reference 1. Byfield, J.E., et al., Pharmacologic requirements for obtaining sensitization of human tumor cells in vitro to combined 5-Fluorouracil or ftorafur and X rays. Int J Radiat Oncol Biol Phys, 1982. 8(11): p. 1923-33. 2. Byfield, J.E., 5-Fluorouracil radiation sensitization--a brief review. Invest New Drugs, 1989. 7(1): p. 111-6. 3. Hiraoka, K., et al., Therapeutic efficacy of replication-competent retrovirus vector-mediated suicide gene therapy in a multifocal colorectal cancer metastasis model. Cancer Res, 2007. 67(11): p. 5345-53.

Imaging exposes the unique voyage of nanoparticles in tumor microenvironment

Randall Toy, Pubudu M. Peiris, Elliott Hayden, Elizabeth Doolittle, Aaron Abramowski, Morgan Tam, Peter Vicente, Jenna Pansky, Andrew Camann, Ruth Keri, David L. Wilson, Efstathios Karathanasis

Case Western Reserve University

Purpose: My research project seeks to design patient-specific therapeutics by exploiting the unique opportunities provided by the engineerability and multifunctionality of nanoparticles. The central thesis of my project is that one nanoparticle does not fit all tumors. Nanoparticle deposition into tumors is a complex process governed by tumor microenvironment characteristics including blood flow, physical gaps of the endothelial fenestrations, interstitial pressures, and microvessel density. Furthermore, the intravascular and transvascular transport of nanoparticles in tumors depends on the relationship of the physical and chemical characteristics of nanoparticles (e.g. size, shape, biochemical functionalization) to the specific characteristics of the microenvironment of each tumor. Using advanced multimodal imaging, a unique cocktail of different nanoparticles can be designed by selecting classes of nanoparticles that 'match' the characteristics of unique tumor regions to maximize nanoparticle interaction with the entire tumor.

Methods: To correlate the physical characteristics of nanoparticles to their in vivo behavior in animal tumor models, non-invasive multimodal imaging was performed at high resolutions to simultaneously obtain anatomical, functional, and molecular information of the tumor microenvironment. Perfusion CT (~152 μm resolution) was used to obtain tumor blood flow maps. Using a micro-CT system and an 'iodinated' nanoparticle contrast agent, we examined the fractional blood volume, microvessel density, and microvascular permeability of a tumor on a voxel by voxel basis at the microcapillary level (20 μm resolution). We employed fluorescence molecular tomography (FMT) to non-invasively measure the time-dependent intratumoral deposition of nanoparticles (liposomes, gold, or iron oxide particles). Co-registration and analysis of 3D-rendered volumes of the multimodal images provided maps of nanoparticle intratumoral deposition as a function of microvascular characteristics.

Results: The in vivo studies showed that 1) tumors display large regional blood flow variability and 2) the deposition of nanoparticles of different sizes (30-200 nm) depends on regional microvascular features. Importantly, we have identified that an optimal nanoparticle size can be tailored to a specific range of blood flows to maximize deposition into that tumor region. Considering tumor regions in terms of blood flow rate, the deposition of nanoparticles followed a size-dependent pattern. The faster flow significantly benefited the extravasation of the larger nanoparticles (100 and 185 nm) by ~2 orders of magnitude. In addition, active targeting benefited primarily smaller nanoparticles in tumor regions with slow flow.

Conclusions: An a priori evaluation of tumor microvascular features can facilitate an 'exclusive' design of a cocktail of different nanoparticles tailored to the regions of a tumor.

The synthesis of the sigma ligand PD14418 and analogs for biological evaluation

Shannon Stittsworth, John R. Lever, Susan Z. Lever
University of Missouri

Cocaine has been shown to have a 15-fold affinity for the sigma-1 receptor over the sigma-2 receptor. Research has shown that certain sigma-1 receptor ligands can block cocaine's behavioral effects. Identifying potent and selective sigma-1 receptor ligands is important in establishing candidates for testing as potential anti-cocaine medications. PD144418 has been shown to have high affinity and selectivity for the sigma-1 receptor and have no significant affinity for any other receptor. Although PD144418 can be purchased in small quantities, we propose the synthesis of the compound in larger quantities as well as a library of analogs. The convergent synthesis of PD144418 using pyridinium, 3-(methoxycarbonyl)-1-propyl and ethanone, 1-(4-methylphenyl)-oxime to form the 3-substituted isoxaxol-5-yl moiety will be attempted. The analogs will be synthesized by replacing the propyl group on the pyridium compound with allyl and iodoallyl groups. The methyl group at the 4 position on the phenyl ring of the oxime compound will be replaced with Fl, Cl, Br and Iodine. The binding affinity and ClogP/LogD7.4 values for each compound will be assessed by in vitro studies in order to determine if the compound is a candidate for testing as an anti-cocaine medication. As of now, there is no data to report but results on the synthesis conditions and yields will be established within the next few months.

Ultraviolet light stimulated cationic lipid charge reversal for siRNA and DNA delivery

Joseph S. Hersey, Dr. Caroline M. LaManna, Dr. Hrvoje Lusic, Dr. Mark W. Grinstaff
Boston University

Purpose: The goal of this project is to develop a cationic lipid delivery system for siRNA and DNA which utilizes a photolabile protecting group that deprotects upon ultraviolet light (UV) exposure (365 nm) yielding neutral or anionic lipids. We hypothesize that spatiotemporal control over the release of nucleic acids can be controlled by triggering this charge reversal once the lipoplexes are within the endosome, leading to more efficient nucleic acid delivery. Herein, we describe the synthesis, characterization, and preliminary in-vitro studies of a library of cationic lipids designed to protect, deliver, and release a genetic payload.

Methods: Synthesis: 1-(2-nitrophenyl)ethyl-based (NPE) cationic lipids with various unsaturated acyl chains (C6, C10, C12, C14) and head groups (lysine-glycine-glycine (KGG), glycine-glycine-glycine (GGG)) were synthesized with a glycerol backbone. Nucleic Acid Binding Affinity and UV Activated Release: An ethidium bromide (EtBr) fluorescence quenching assay determined the DNA and siRNA binding affinities and UV (?= 365 nm, 30 minutes) activated release for the photo-active lipoplexes. Quantitative Cellular Uptake: Cellular uptake in Chinese hamster ovarian (CHO) cells was determined using lipoplexes formed with rhodamine-labeled DNA and using flow cytometry to quantify the rhodamine within the cells.

Results: The charge of the head group and the length of the hydrocarbon chains directly affect the binding affinities and UV induced release properties for nucleic acid payloads. The divalent KGG-C10-NPE liposomes had the strongest affinity for DNA and siRNA (1.85E6 M-1 and 2.62E6 M-1, respectively) and comparable payload release as the KGG-C6-NPE liposomes (~58%). The KGG-C14-NPE liposomes had good binding affinities (~1.3E6 M-1 and 2.0E6 M-1 for DNA and siRNA, respectively); however, they had poor release properties upon UV exposure (7.44%±2.28 and 14.56%±0.85, respectively). The lipid with a monovalent head group, GGG-C10-NPE, had poor binding affinities for both DNA and siRNA (4.41E4 M-1 and 6.13E4 M-1, respectively) despite having the same chain length as KGG-C10-NPE. Optimal cellular uptake was obtained using divalent lipids with C10 and C14 chain lengths, where ~90% uptake was achieved within 3 hours.

Conclusions: We have successfully synthesized and characterized a library of cationic lipids that are activated by UV light to release a genetic payload. The head group charge dictates the order of magnitude of the binding affinities and payload release, while the chain lengths allow for fine control over these properties. Future work will explore the effects of the head groups and chain lengths on endosomal escape and transfection efficiency for nucleic acid payloads.

Development of a Physiologically Relevant In Vitro Model of the Blood-Brain Barrier

Jack D. Wang, Nick J. Douville, Shuichi Takayama, Mohamed E.H. ElSayed
University of Michigan

PURPOSE: The endothelial cells lining the capillaries that supply the brain with oxygen and nutrients present a highly regulated transport barrier known as the blood-brain barrier (BBB). These cells are characterized by thick cell membranes, minimal endocytic vesicles, and highly organized tight junctions that restrict molecular diffusion across the paracellular space. Several groups have cultured primary and immortalized brain capillary endothelial cells to develop an in vitro model that mimics the BBB for the purpose of screening transport properties of new drug molecules designed for treatment of CNS disorders. However, these in vitro models failed to mimic the restrictive properties of the BBB due to the formation of "loose" tight junctions, lower expression of specific carriers and transporters, and limited cell viability. We report the development of a new 3D in vitro model of the BBB that is characterized with improved endothelial cell polarization, enhanced formation of tight junctions, and minimal dilution of secreted chemical factors.

METHODS: Layered microfluidic channels were fabricated using standard soft lithography procedures. The PDMS mortar layers were designed as a two-layer system similar to conventional transwells that enables permeability studies on the cell monolayers within the system. After curing, the layers are glued using PDMS and toluene mixture at 3 (PDMS) : 2 (toluene) weight ratio. The layered channels were then exposed to plasma oxidation for 10-15 minutes.

RESULTS: This new 3D model proved to be 10-fold more restrictive compared to conventional endothelial monolayers evaluated with of [14C]-mannitol permeability; transendothelial electrical resistance (TEER) also demonstrates at least 5-fold increase. In addition, we found comparable levels of P-glycoprotein expression in both the transwells and the layered microfluidic channels. Our results collectively indicate that we have successfully developed a new in vitro model of the BBB that mimics its physiological barrier properties in vivo.

CONCLUSION: This project utilizes microfluidic devices to develop a 3D, physiologically relevant in vitro model of the BBB, which allows us to better understand the mechanisms involved in regulating drug transport across the blood-brain barrier. Furthermore, the physiological mimicry of the proposed model will allow for accurate in vitro/in vivo correlation of transport data, which will expedite the development of new drug molecules designed for treatment of CNS disorders.

Biophysics

Formin regulation at the barbed ends of actin filaments

Jeffrey P Bombardier, Bruce Goode, Jeff Gelles
Brandeis University, Dept of Biochemistry

The actin cytoskeleton is a dynamic filament network responsible for cellular cytokinesis, intracellular transport, and cellular motility. Actin does not act alone: cells contain numerous actin binding proteins that control the behavior of actin monomers and actin filaments. Two such proteins are capping protein (CP) and formins. CP is a ubiquitously expressed protein in humans. It binds to the barbed (fast growing) ends of actin filaments and stops the addition of actin monomers to the ends. CP is known to play an important role in cellular motility; knockdowns of CP severely limit the ability of cells to move directionally. Formins have multiple biochemical activities which may include actin filament nucleation, acceleration of filament elongation (in the presence of the cofactor profilin), and protection of the barbed end from CP. Formins contribute to have multiple cellular processes, including motility. Given the antagonistic effects of formin and CP on actin filaments in vitro, and reported synthetic interactions, between formin and CP genes in vivo, we hypothesize that formin and CP either directly compete for or mutually destabilize each other's binding to the barbed ends of actin filaments. The first hypothesis predicts that a formin must first dissociate from the barbed end before CP can bind. The second predicts formation of a ternary complex of formin, CP, and the filament end from which formin then dissociates. To distinguish these possibilities, we are performing quantitative single-molecule total internal reflection fluorescence (TIRF) microscopy studies using fluorescently labeled formin and actin filaments in separate colors. We directly observe single formin molecules "surfing" along with barbed ends of actin filaments as they grow. In the presence of CP, the filament stops growing but the formin remains attached to the filament barbed end. This behavior virtually never happens in the absence of CP, suggesting that it is caused by the formation of a heretofore unknown ternary complex that blocks filament polymerization. Future work involving three-color TIRF with labeled CP is underway and should give more insight into the nature and dynamic behavior of the ternary complex.

PGC-1 alpha affects action potential conduction and morphology in neonatal rat ventricular myocytes

Geran Kostecki, Gordon Tomaselli
Johns Hopkins University

Purpose: Recent analysis of gene microarray data from failing and non-failing human heart samples has shown a surprisingly strong correlation between expression of PGC-1a and certain ion channels. (Barth, et al. unpublished data) A recent study (Chen, et al. 2010) confirmed that overexpression of PGC-1a upregulates ATP2A2 which codes for sarco/endoplasmic reticulum Ca2+-ATPase (SERCA), resulting in an increased rate of calcium clearance. However, for other ion channels (e.g. SCN5A, GJA1), whether changes in levels of channel mRNA are actually caused by alterations in PGC-1a functional expression and produce a change in electrical phenotype remains unclear.

Methods: Confluent cell monolayers were produced by isolating and culturing NRVMs on 21mm fibronectin-coated coverslips as previously described. (Sekar, et al. 2007) 24 hours after plating, CAG.PGC-1a.IRES.GFP lentivirus was added to the monolayers at various MOI's (0,8,24,80) for 24 hours to facilitate PGC1-a expression. Six to nine days after plating, they were optically mapped using the voltage-sensitive dye di-4-ANEPPS, as previously described. (Lim, et al. 2006) Using custom MATLAB software, conduction velocity, action potential duration, and maximum upstroke and repolarization rates for each monolayer were calculated.

Results: Virus dose-dependent changes in action potential parameters were found across pacing cycle lengths. Addition of virus at an MOI as low as 24 significantly decreased conduction velocity, maximum upstroke rate, and maximum repolarization rate, while increasing action potential duration at both 30 and 80% repolarization .

Conclusion: The results of this study demonstrate that metabolic state and electrical excitability are reciprocally coregulated. PGC-1a is a transcription factor that acts as a control point for cellular metabolism that shifts the cell's energy source from glucolysis to fatty acid oxidation. By upregulating PGC-1 alpha in fatty acid free medium, we shifted cellular metabolism to an energy source that was unavailable, inducing metabolic stress. Decreased conduction velocity and maximum upstroke rate suggest a decrease in sodium channel conductance, while increased action potential duration increases the refractory period of cells, decreasing their maximum pacing rate. Decreased repolarization rate suggests a decrease in potassium channel conductance. All of these effects work to decrease the rate of flow of ions across the cell membrane. Since energy from metabolism is required to pump these ions across the cell membrane in order to maintain homeostasis, the cell may cause these changes to decrease the metabolic load of excitation, and concommitant contraction, during times of metabolic stress.

Single-Turnover Stopped-Flow Fluorescence Applied to ClpAP Catalyzed Polypeptide Translocation

Justin M. Miller, Tao Li, Aaron L. Lucius
University of Alabama at Birmingham

Many macromolecules can perform functions within the cell by converting the energy of ATP into mechanical work. One such example of this can be found in the ATP-dependent protease from Escherichia coli, ClpAP, which is assembled from two distinct enzymes, a protein unfoldase, ClpA, and a protease, ClpP. The biologically active complex functions through a coordinated action in which ClpA is responsible for enzyme catalyzed protein unfolding and ATP-dependent polypeptide translocation, while ClpP will proteolytically degrade polypeptide substrates that have been translocated into its central cavity. Depending upon the ratio of [ClpA] to [ClpP], ClpA can exist either as a free hexamer or associated as a hexamer with one or both sides of tetradecameric ClpP. All three of these assembly states are competent for polypeptide translocation. It is unknown as to whether the different assembly states of ClpAP process their substrates via the same kinetic mechanism or if each species is unique in regards to how substrate is degraded. In order to attempt to answer this question, single turnover stopped-flow fluorescence techniques have been used to elucidate relevant kinetic parameters such as the kinetic step-size of polypeptide translocation, macroscopic rate constants, and microscopic rate constants as a function of the ratio of the molar ratio, [ClpA] to [ClpP]. Through the observation of trends in observed kinetic parameters as a function of the molar ratio, we show here that the polypeptide translocation activity must be allosterically modulated through interactions with the ClpA molecular motor. We also describe here an estimate of affinity of the interaction of ClpA and ClpP to be approximately 0.95 nM, and show that the ClpAP complexes translocate polypeptide identically with an overall rate of translocation of 34.3±0.75 AA s-1, whether a one or two ClpA hexamers are associated with ClpP.

Modeling Stretch-Induced Release of Molecules in the Actin Cytoskeleton

John Kang, Kathy M. Puskar, Philip R. LeDuc, Russell S. Schwartz
Carnegie Mellon University

PURPOSE: Recent literature provides strong evidence for a causative link between the mechanical stretching of cytoskeleton and the release of signaling molecules. Understanding the link between the mechanical input, the corresponding morphological changes in the actin cytoskeleton, and the final signaling molecule release is a poorly understood yet very significant problem in the field of mechanotransduction. Here, we present a coarse-grained actin network model that simulates architectural changes in the network in response to external mechanical stimulus to understand the interplay between actin network mechanics and resulting biochemical signaling. We develop a coarse-grained actin network model and examine the intrinsic angle geometry changes under mechanical stimulation.

METHODS: We have previously examined actin network mechanics with a derivative of this model. The network is created with a circular solution space that is considered fixed to an underlying substrate at uniformly placed perimeter nodes representing focal adhesions. Filaments are formed by crosslinking opposite focal adhesions on the periphery. Intersections formed by crosslinking represent molecular linking of actin with affiliated attached molecules (e.g. filamin A and FilGAP) at each of the four angles created by that intersection. Stretch is simulated by moving the upper nodes while holding the bottom nodes stationary and balancing forces on the remaining nodes.

RESULTS: As stretch is applied to the network, the intersecting angle distribution transitions from a more peaked to a flatter distribution while still being centered at 90º. We also examined the difference in the stretched angle relative to the non-stretched angle ("delta angle"), and observe a shift in distribution as additional stretch is applied. At 1% stretch, the delta angles are small with almost no angle changes greater than 5 degrees, but at higher levels of stretch, the observed delta angle distribution is almost uniform. These histograms reflect similar results to simulations performed by Ehrlicher et al. on their experiments even though their simulations had different overall morphologies and boundary conditions .

CONCLUSION: These results are being incorporated into molecular release models that represent a potentially versatile platform for examining the biophysical interactions that link mechanical stimulus at the cellular level to response at the protein level and may underlie strain-induced signaling mediated by the cytoskeleton.

Optogenetics and Sleep

Peter Dahlberg, Sean Gibbons, Vu Quoc Dinh, Kristen Hitchcox, Daniel Kerr, Boleslaw Osinski, Guillermina Ramirez, Adam Hammond
University of Chicago

This body of work is the product of a first year lab course conducted by the University of Chicago's Graduate Program in Biophysical Sciences. The lab course gives incoming students an understanding of the state of knowledge in biophysics, teaches grant writing and research presentation skills, and allows the students to develop as independent researchers. This year the course focused on student-led investigations of the neural basis of sleep in the model organism, Caenorhabditis elegans. To probe the neural network, we expressed Channelrhodopsin in neurons associated with the sleep state, Lethargus. We were able to excite these key neurons by using a high-resolution projector as the illumination source for an inverted microscope. Quantifying the action of these neurons allows for a more comprehensive understanding of sleep.

Threading a Protein Sequence onto its CryoEM Density Map

Charles Greenberg, Keren Lasker, Andrej Sali
University of California, San Francisco

Purpose: Advances in electron microscopy allow for structure determination of large biological machines at increasingly high resolutions. A key step in this process is interpreting the density to the highest resolution possible. To date, structural interpretation of a density map requires a reliable fit of a homologous structure; however, in many cases such a structure is not available.

Methods: We present MultiThread, a method for threading (assigning) a protein sequence onto an intermediate resolution cryo-EM density map. Possible assignments are sampled by looking for linear paths in a density map discretized to "CA" regions. If no further information is provided, the method produces a set of linear paths through the density map, each of which is a possible CA trace of the target sequence. In many cases, however, templates for parts of the sequence, secondary structure prediction, and additional information, such as cross-linking, are available. These data are incorporated into the sampling procedure to generate a smaller and more precise ensemble of models.

Results: We validated the method using a set of experimentally derived, previously published EM data of macromolecular complexes at varying resolutions within 6-10Å. We were able to recover the fold for most subunits using a template coverage of of 10-100%. These results demonstrate MultiThread's ability to determine structures when available prior data are limited.

Conclusion: Knowledge of the architecture of a macromolecular complex is essential in understanding the physical basis for its function. MultiThread addresses a critical need in determining such structures: modeling (at least to pseudo-atomic level) when limited homology data are available.

Lef1-mediated up-regulation of Epac1 expression in Chronic Lymphocytic Leukemic Cells

Loren Brown, Fiona Murray, Lingzhi Zhang, Paul Insel
University of California, San Diego

Purpose: Exchange protein activated by cAMP 1 (Epac1), a guanine-nucleotide exchange factor for the small G-protein Rap1, contributes to anti-apoptosis and other cAMP-mediated actions. Limited previous studies have noted increased expression of Epac1 in chronic lymphocytic leukemia (CLL) B-cells. We hypothesized that this increase in Epac1 expression results from increased transcriptional activity of the Epac1 gene and that this increase contributes to the anti-apoptotic phenotype of CLL cells.

Methods: PBMC Isolation: Blood was collected from healthy donors and CLL patients after informed consent. PBMC were isolated by density-gradient centrifugation using Ficoll-Paque (Amersham Biosciences), washed, suspended in FCS containing 10% DMSO. Isolation of B Cells: B cells were isolated from PBMC by using either Dynabeads CD19 pan B or a Dynal B cell negative isolation kit (Invitrogen), which resulted in 90% CD19 cells, as assessed by flow cytometry. Real-time RT-PCR: Total RNA was isolated by use of Trizol. Luciferase Assays: Activity was measured with the DLR Assay System (Promega) on a Turner 20/20 Luminometer.

Results:We assessed peripheral blood mononuclear cells (PBMC) and found that Epac1 mRNA (real-time PCR) and protein (immunoblotting) are up-regulated ~70 fold and ~10 fold, respectively, in CLL patients compared to controls. Deletional analysis of the 2kb promoter region directly upstream of the Epac1 start site revealed that the region necessary for basal expression is located within 100 base pairs of the transcriptional start site. Overexpression of Lef-1, a transcription factor up-regulated in CLL, increased activity of a Epac1 promoter luciferase reporter in a CLL cell line but not a normal B-cell cell line. Stimulation of CLL cells with Wnt3 did not increase activity of the Epac1 promoter reporter but increased activity of a Lef1 luciferase reporter. These results, which indicate that Epac1 is up-regulated in CLL patients and that Lef1 regulates Epac1 by a non-cannonical pathway that is unique to the CLL B cells, define a transcriptional mechanism for the regulation of Epac expression in human disease.

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Systems Biology

Motile Droplets: Active 2D Nematics on a Spherical Surface

Stephen J DeCamp, Tim Sanchez, Daniel T. N. Chen, Zvonimir Dogic
Brandeis University

We study diverse phenomena that emerge from extensile bundles composed of microtubules, motor proteins, and a depletion agent. Upon confinement in an emulsion droplet, the system of microtubules self-organizes into an active 2D nematic at the oil-water interface where the creation, propagation, and annihilation of defect pairs is observed. These interfacial defect textures generate an active flow on the droplet surface which leads to a striking new form of self-motility of the droplet.

Interdisciplinary Approach to Discovering Novel Natural Products from Cave Bacteria Communities

Ara Kooser, Dr. Charles E. Melancon, Dr. Diana Northup
Univeristy of New Mexico

Microbial natural products are an excellent source of new drugs, drug leads, and small molecule biological probes. Natural products may also mediate ecological relationships among microbes in complex environments. Caves are complex ecosystems harboring many novel species of microbes from prolific natural product producing phyla, and thus are an ideal habitat for discovering novel natural products. We use bioinformatics coupled with culturing methods; gene and genome sequencing; and mass spectrometry to examine cave bacterial communities for natural product production. In this project, we will use a combination of geomicrobiological, bioinformatic, and chemical approaches to discover novel natural products made by cave bacteria.

Cluster analysis of Prochlorococcus gene abundance from widely distributed oceanic samples

Alyssa Kent, Adam Martiny
University of California, Irvine

Prochlorococcus, the smallest known photosynthetic bacterium is ubiquitous in the ocean's surface layer despite variation in environmental conditions. Due to its abundance and wide distribution, identifying genes related to specific ecological niches is a major step in understanding its persistence. In order to identify distributions of Prochlorococcus genes, we used molecular data from the Global Oceanic Sampling Expedition to identify sample sequences highly similar to known Prochlorococcus genes. Coupling the genetic information with abiotic factors informed us about how Prochlorococcus genes are distributed. Samples were hierarchically clustered based on Bray-Curtis similarity of orthologous gene abundance. Analogous to gene expression profiling studies that identify groups of genes expressing similarly under specific conditions, we profiled orthologous gene group abundances based on different environmental parameters such as temperature and levels of nitrate, phosphate, and iron to identify correlated gene groups. Identifying the relationship between groups of orthologous genes and the abiotic factors controlling their expression gives us a better understanding of the components controlling the gene abundance of sampled Prochlorococcus.

Effects of Fc Density and Microparticle Size on Macrophage and Complement System Activation

Patricia M. Pacheco, David White, D.V.M., Ph.D., DACVM, Todd Sulchek, PhD
Georgia Institute of Technology

Introduction: Macrophages and the complement system are vital components of the humoral immune response that can be modulated by extrinsic factors, such as a functionalized biomaterial. The Fc fragment of IgG interacts with both macrophages and the complement system through receptor mediated phagocytosis and cascade activation respectively, but the impact of the surface density of this activating ligand is not yet fully understood. Hence our objective is to use functionalized microparticles of varied Fc densities and sizes to modulate the macrophage and complement system response.

Methods: We used polystyrene microparticles with diameters of 0.5, 1, 2, 3, and 4.5 um and functionalized each microparticle with varying amounts of Fc. These microparticles were first opsonized with bovine serum albumin (BSA) through absorption and then incubated with anti-BSA IgG. For our phagocytosis studies, we allowed the functionalized microparticles to incubate with RAW264.7 macrophage cells and then use for flow cytometry and fluorescent microscopy for analysis. A Griess assay was used to determine the amount of nitrite produced by the macrophages as an indicator of their inflammatory state. To determine complement activation, functionalized microparticles were incubated with fresh human serum and the resulting terminal complement complexes were quantified using the Quidel CH50 EIA kit.

Results: We were able to successfully vary the Fc density on each particle size as was confirmed by measuring the fluorescent intensity of a fluorescently labeled secondary antibody. The attachment study showed no significant dependence on Fc density and the only microparticle size that attached at a significantly higher level was the 0.5 um microparticle. However, the internalization assays showed a strong dependence on both the size and Fc density of the microparticle. Size and Fc density was also significant in determining complement system activation. Only the smaller 1 um microparticle functionalized with high Fc density was able to successfully activate the complement system. Finally, while Fc density and size did not seem to be strong predictors for the amount of NO2 produced, the addition of functionalized microparticles did impact the NO2 production.

Conclusions: The dependence of phagocytosis on Fc density for only small particles indicates an alternate pathway for larger particles. As an increased density of Fc is not significant for attachment, this suggests that a higher Fc density affects the kinetics of internalization. The dependence of smaller size and high Fc density for complement system activation may be biologically significant as microbes are typically 1-2 um.

ATP-Mg salt and Oxygenated Perfluorocabon Protects the Intestinal Mucosa during Gut Ischemia

Marisol Chang, Pedro Cabrales, Geert Schmid-Schönbein
University of California, San Diego, Dept. of Bioengineering,

Intestinal ischemia encountered in clinical conditions, such as hemorrhagic shock, sepsis and cardiovascular surgery results in many different systemic responses leading to multiple organ failure. The mechanisms by which intestinal ischemia causes these deleterious effects are still not well characterized; we have identified that during early periods of gut ischemia disruption of mucin in the mucosal barrier is accompanied by entry of digestive enzymes across the intestinal wall. In this study we investigate how events characteristic of ischemia such as ATP and oxygen depletion are involved in the mechanism resulting in disruption of the mucosal barrier, we also investigate the use ATP-Mg salt or oxygenated perfluorocarbons as potential treatments for intestinal ischemic injury. We used a rat model of intestinal ischemia by occlusion of the superior mesenteric and celiac arteries (SAO) for 30min; animals with SAO were treated with either a luminal injection of ATP-Mg salt or with oxygenated perfluorocabon solution, a sham group with the treatment but without SAO was also studied. After the surgery, a jejunal sector was dissected and processed for analysis. It was determined via western blot that during SAO mucin 2 (an isoform that covers the epithelial cells) is degraded and mucin 13 (a membrane bound isoform) is fragmented as seen in appearance of additional bands not observed in the sham group. Treatment with either ATP-Mg salt or oxygenated perfluorocarbon attenuated mucin 2 and mucin 13 disruption. It is concluded that during early periods of ischemia the mucosal barrier is disrupted allowing entry of digestive enzymes and other cytotoxic mediators, this study identifies potential treatments for intestinal ischemic injury and sheds light into the possible mechanism of gut-derived shock.

Assessing Phenotypic Convergence in Genetically Divergent Subsets of Heat-tolerant Escherichia coli

Shaun Hug, Brandon Gaut, Anthony Long
University of California, Irvine

Studies of evolution, both in the wild and in the laboratory, have become much more controlled and precise in recent decades, but they often face evolutionary biology's equivalent of the "three-body problem" that plagues physicists: the behavior of systems with two interacting components can be characterized quite well, but in systems with three interacting components, behavior is much harder to understand and predict. In evolutionary biology, interactions between genotype, phenotype, and fitness all affect the course of adaptation, and even if two of these can be well described in a particular system, precise control or measurement of the third is often elusive. Previously, 115 populations of Escherichia coli were evolved from a common ancestor adapted to a 37°C environment. For 2,000 generations, these 115 lines were subjected to an environmental pressure of 42.2°C, and at the end of the evolution period, single clones from each line had their genomes sequenced. Most mutant lines fell into two statistically distinct groups: those with mutations in rpoB, which codes for the ß subunit of RNA polymerase, and those with mutations in rho, which codes for a major transcriptional termination factor. Very few mutants possessed mutations in both, indicating strong epistasis between these gene changes and two divergent genetic pathways to adaptation. With genotypes well characterized, the next step to understanding adaptation is phenotypic characterization. RNA-seq was performed on subsets of the rho- and rpoB-type mutants, with the resulting data on mRNA levels interpretable as a gene expression phenotype. Phenotypic microarrays, 96-well plates that assess the ability of bacteria to metabolize and tolerate various compounds, were also performed on these subsets to obtain further phenotype data. These two types of phenotype measurements have been compared with each other, both within and between mutant subsets, to assess whether the two divergent genetic pathways lead to convergence or divergence at the phenotypic level. Future work will utilize fluorescent competition assays to precisely characterize fitness differences between mutants, with the ultimate goal of putting together all three pieces of evolutionary biology's "three-body problem" into a cohesive characterization of adaptation in this system.